reactivity and greater selectivity can be obtained by adding a reduced amount of $AlCl_3$.
10. The chemical shifts of these 4 $^1$H-NMR peaks, which have been assigned to 4 different compounds, are consistent with the our previous publication (for a detail, see Table 3 in ref. 11).
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15. Calculations have been performed with GAUSSIAN-92. Optimized structures have been determined at the HF/3-21G* and HF/6-31G* level. The TS was located by the eigenvector following routine (OPT=(TS, EF)), and confirmed by the frequency calculation (TS have only one imaginary frequency; $-619.8i$ cm$^{-1}$ at HF/3-21G* and $-593.4i$ cm$^{-1}$ at HF/6-31G*). Single-point energy calculations have been performed at the MP2, MP3, and MP4 levels to incorporate the electron correlation effect. Corrections also have been made for zero-point vibrational energies. Calculations have been run on a CRAY Y-MP2 E/232.
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18. Since these values are from the bending of H-Si-H angle, the estimated ring strains may have errors which come from the comparison between the H-Si-H and C-Si-C angle changes. However, the approximate force constant (0.38 mdyn.A°/rad$^2$) for the H-Si-H angle bending is smaller than the one (0.48 mdyn.A°/rad$^2$) for the C-Si-C angle bending. Therefore, the actual ring strains will be greater than our values.

## Characterization of Conformational Changes in [d(ACGTATACGT)]$_2$-echinomycin and [d(ACGTTAACGT)]$_2$-echinomycin complexes by proton NMR studies

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*Received October 4, 1993*

Echinomycin derived from streptomyces is cyclic depsipeptide containing two planar quinoxaline rings that bisintercalate into DNA[1,2]. Echinomycin has antitumor activities and is now being used in phase II clinical trials for the treatment of human tumors[3]. Recently, a series of crystal and solution structure determinations of the complexes between echinomycin and DNA oligonucleotide fragments have been carried out in order to visualize how a drug can be accommodated by the DNA double helix[4-6]. According to the crystal structure of echinomycin complex with DNA hexamer [d(CGTACG)]$_2$, two echinomycins bind to each DNA duplex with the quinoxaline rings bracketing the CpG steps[4]. A remarkable feature of this structure is that the central A·T base pairs are Hoogsteen base paired. Using specifically deuterated DNA hexamer we also proved that the Hoogsteen base pair formation detected in X-ray crystallographic analysis was definitely retained in solution[7,8].

At this situation, it is of interest to ask how many Hoogsteen base pairs are formed near the binding sites when echinomycins bind to a DNA oligonucleotide in which the CpG binding sites are separated by more than two A·T base pairs. For example, in a complex between a DNA decamer [d(ACGTATACGT)]$_2$ and echinomycin, is it possible to form the Hoogsteen base pairs in the four central A·T base pairs? In order to address this question, we have examined two DNA decamers, [d(ACGTATACGT)]$_2$ and [d(ACGTTAACGT)]$_2$ in which echinomycin binding sites are separated by four base pairs.

The decamers, [d(ACGTATACGT)]$_2$ and [d(ACGTTAACGT)]$_2$ were synthesized on an Applied Biosystems DNA synthesizer using β-cyanoethyl phosphoramidite chemistry on a 2 μmole scale. The oligonucleotides were purified by Sephadex G-25 gel filtration column chromatography. Echinomycin was a gift from National Cancer Institute, USA. A saturated echinomycin-DNA complex of two drugs per DNA decamer was formed by adding 2 equivalents of echinomycin in methanol to the DNA sample in the NMR tube as reported previously[8]. All NMR experiments were done on a Bruker AMX-500 spectrometer.

A comparison of the imino and aromatic spectra of the two decamers, [d(ACGTATACGT)]$_2$ and [d(ACGTTAACGT)]$_2$ and their echinomycin complexes are shown in parts A and B of Figure 1, respectively. The imino protons have been assigned from a combination of NOE and temperature dependence measurements. The G3 and G9 imino resonances of both decamers shift upfield approximately 1 ppm when echinomycins bind. The T10 imino resonances also shift upfiled approximately 1 ppm at 1℃ (data not shown). These large upfield shifts are due to intercalative drug binding. The central T imino resonances of both decamers, however, shift only 0.2 ppm upfield or do not show any changes in chemical shift values. Previous NMR results showed that the central T imino resonance of the DNA hexamer [d(CGTACG)]$_2$-echinomycin complex were upfield-shifted by 1 ppm and A·T Hoogsteen base pairs were formed[8]. Therefore the central A·T base pairs of the present two decamers probably would not be Hoogsteen base pairs. As shown in Figure 1, the central T imino resonances of the [d(ACGTATACGT)]$_2$-echinomycin complex are much broader than those of the free DNA or [d(ACGTTAACGT)]$_2$-echinomycin complex, indicating that echinomycin binding destabilizes the base pairs in 'TATA' sequence. It is interesting that the imino resona-

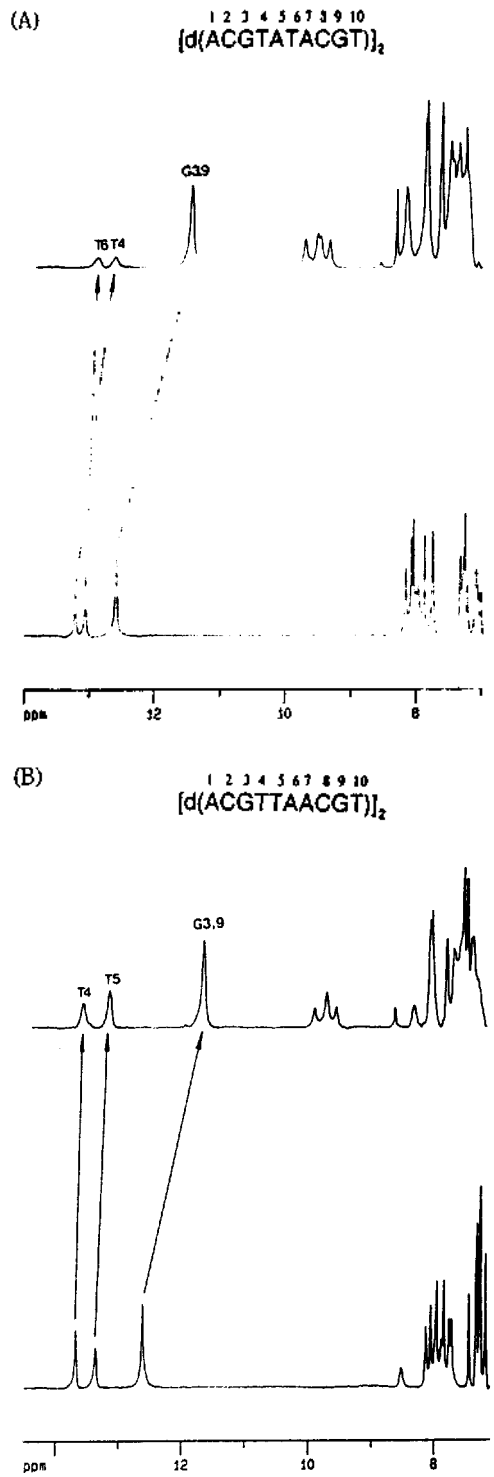


**Figure 1.** (A) The 500 MHz proton NMR spectra of imino and aromatic region of the echinomycin-[d(ACGTATACGT)]$_2$ complex (top) and free DNA, [d(ACGTATACGT)]$_2$ (bottom) in 90% $H_2O$/10% $D_2O$, 200 mM NaCl, pH 6.5 at 20℃. Spectra were acquired by using the jump and return pulse sequence[10] ($90°'_x - \nabla 90°_{-x}$) to suppress water resonance. $\nabla = 80$ μsec was used. Arrows indicate the changes in chemical shift positions of imino proton resonances; (B) The 500 MHz proton NMR spectra of imino and aromatic region of echinomycin-[d(ACGTTAACGT)]$_2$ complex (top) and free DNA, [d(ACGTTAACGT)]$_2$ (bottom), otherwise similar to (A).

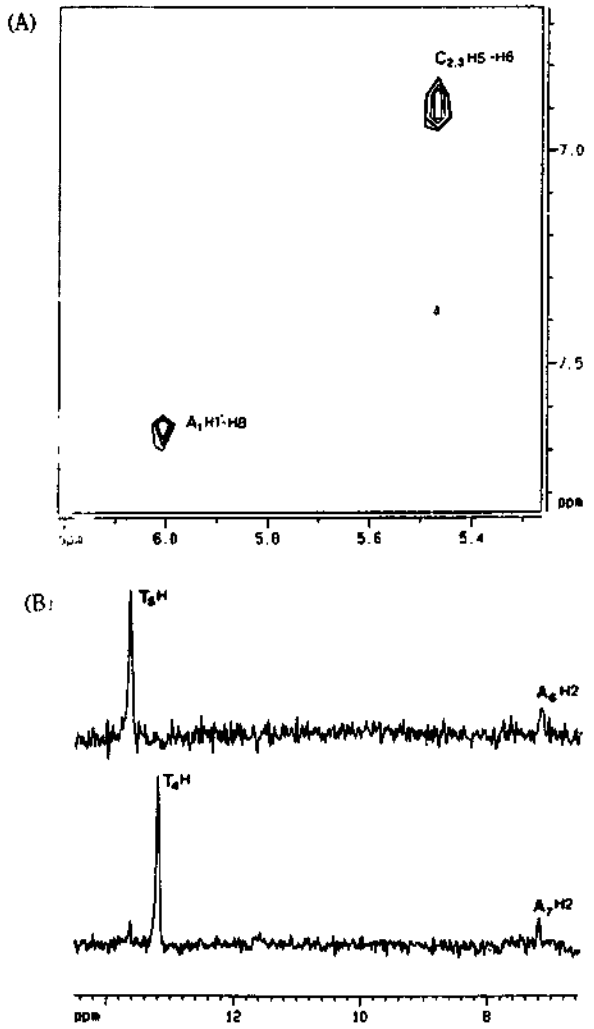


**Figure 2.** (A) Expanded region of the 500 MHz protom NOESY spectrum of echinomycin-[d(ACGTATACGT)]$_2$ complex in $D_2O$, showing the aromatic resonances and their cross peaks to the H1' region at 20℃ and $\tau_m = 50$ ms. The sample was in 200 mM NaCl, pH 6.5 in $D_2O$. The residual water was suppressed by irradiating the water resonance during the 1.3 second recyclic delay. 512 $t_1$ blocks of 2 K points and 32 scans were acquired with a spectral width of 5000 Hz in both dimensions; (B) Cross sections of the 500 MHz proton NOESY spectrum of echinomycin-[d(ACGTTAACGT)]$_2$ complex, 200 mM NaCl, pH 6.5, in 90% $H_2O$/10% $D_2O$ at 20℃ and $\tau_m = 150$ ms, showing the cross peaks of T4 (top) and T5 (bottom) imino protons. The T imino-AH2 cross peaks are indicated.

nces of the two decamers with small variations in their sequences show the quite different dynamical behaviours.

Figure 2(A) shows a short mixing time (50 ms) NOESY spectrum of the [d(ACGTATACGT)]$_2$-echinomycin complex. We are able to observe a very strong cross peak between A1H8 and its own H1' which confirms that A1 is in the *syn* conformation and terminal A·T base pairs are Hoogsteen base paired. In contrast to the terminal A·T base pairs, there is no clear evidence that the central A·T base pairs are Hoogsteen base paired. Gilbert and Feigon[9] suggested that these could be either a Watson-Crick base pair and an open

state or a Hoogsteen base pair by their NMR results. Figure 2(B) illustrates a cross-sectional representation of the NOE connectivities in the imino and aromatic region of the two-dimensional NOESY spectrum of the [d(ACGTTAACGT)]$_2$-echinomycin complex. The strong intra-base pair NOE was observed between T imino and AH2 proton resonances. If the central A·T base pairs are standard Watson-Crick A·T base pairs which have an adenosine H-2 proton adjacent to the imino proton, a strong intra-basepair NOE should be observed. Therefore, in contrast to the results obtained with [d(ACGTATACGT)]$_2$ or [d(CGTACG)]$_2$, these data clearly confirm that the central A·T base pairs in the DNA oligomer with 'TTAA' sequences maintain Watson-Crick base pairs even when echinomycin binds.

In summary, we have demonstrated that the Hoogsteen base pairs are not propagated one base pair away from the binding site and structural changes induced when echinomycins bind are sequence specific. More detailed structural studies of the two decamers are under progress.

**Acknowledgement.** We thank Dr. Karl P. Flora (National Cancer Institute, USA) for providing the echinomycin, and gratefully acknowledge the Basic Science Research Institute Program, Ministry of Education (BSRI-93-316) for financial support.

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## Convenient Method for the Preparation of Psoralen Cross-Linked DNA Oligomer

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Psoralens are linear furocoumarines that photochemically alkylate nucleic acids and have been used as intercalating DNA binding drugs in the photochemotherapy of skin diseases.[1] They have also been used as probes for nucleic acid structure and function.[2] Model studies for psoralen cross-linking with DNA have been suggested that the photochemical reactions take place at the 3, 4 or 4', 5' double bonds of the psoralen with the 5, 6 double bond in pyrimidines.[3] Chart 1 shows the structure of cross-linked adduct between thymidine and 4'-aminomethyl-3,5',8-trimethylpsoralen (AMT).[4] Thymidine is the preferred site for monoadduct formation, and psoralen cross-linking occurs at 5'-TpA-3' sites in DNA.[5] In spite of all the work on the monoadduct structure, the solution-state structures and properties of psoralen cross-linked DNA as a function of sequence are still not known. This has been due in large part to the lack of general methods for the preparation of psoralen crosslinked DNA in sufficient purity and quantity for detailed NMR studies. Generally, the denaturating polyacrylamide gel electrophoresis (PAGE), followed by the elution of the DNA from gel slices, is the most common method for the separation of photoadducted DNA oligomers. However, this method is obviously insuitable for the production of large quantities of pure psoralen cross-linked DNA molecules required for NMR studies. Recently, the method for the large-scale synthesis of the photoadducted DNA using laser and HPLC has been reported.[6,7] This method is also not easily used because a laser equipment as a light source should be prepared. To this end, we would like to report a convenient methodology which involves a traditional light source and simple column chromatography for the complete separation of the pure psoralen-adducted DNA oligomers.

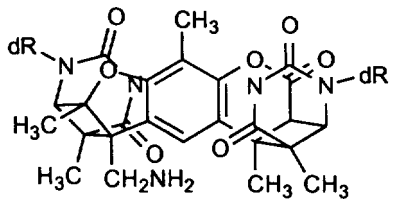
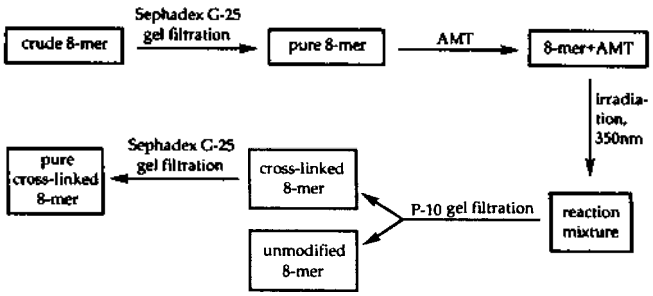



**Chart 1.**




**Scheme 1.**

Scheme 1 shows a flow chart of AMT cross-linked oligonucleotide synthetic scheme. The DNA oligomer, d(GGGTACCC), was synthesized on an automated applied biosystems DNA synthesizer using β-cyanoethyl phosphoramidite chemistry on a 10 μmole scale and deprotected using ammonia and 80% acetic acid. The oligonucleotide was purified by Sephadex G-25 gel filtration column chromatography. The purified oligonucleotides (100 mg) and AMT (1.76 mg) toge-