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### Communications

#### NMR Assay for Hoogsteen Base Pairing in Echinomycin Complex with Specifically Deuterated DNA Hexamer

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Echinomycin is a cyclic octadepsipeptide antibiotic with two quinoxaline rings (Chart I) that bisintercalate into DNA. According to the crystal structure of echinomycin with the DNA hexamer  $[d(\text{CGTACG})]_2$ , two echinomycins bind to each DNA duplex with the quinoxaline rings bracketing the CpG steps.<sup>1</sup> A remarkable feature of this structure is that the A · T base pairs are Hoogsteen base paired (see Chart II). Previous NMR studies of echinomycin- $[d(\text{ACGTACGT})]_2$  complex have tried to show that A · T Hoogsteen pairs are formed in solution at low temperature.<sup>2,3</sup> One of the characteristic features in A · T Hoogsteen base pair is the short distance between thymidine imino proton and adenosine base proton at 8 position. This can be detected by nuclear Overhauser enhancement (NOE) experiment. However, adenosine H-8 proton resonance is easily overlapped with quinoxaline ring proton resonances of drug, and these are the cases for the previous NMR experiments.<sup>2,3</sup> Therefore, some ambiguities still remain unresolved.

To address the question of Hoogsteen base pair formation, we have investigated complex formation between echinomycin and the specifically deuterated DNA hexamer  $[d(\text{CGTACG})]_2$  using NOE method. Normal hexamer  $d(\text{CGTACG})$  and adenosine H-8 deuterated hexamer  $d(\text{CGTA(D)CG})$  were synthesized *via* solid phase method as reported previously.<sup>4</sup> Echinomycin was a gift from National Cancer Institute, USA. A saturated echinomycin-DNA complex of two drugs per DNA hexamer was formed by adding 2 equivalents of echinomycin in methanol to the DNA sample in the NMR

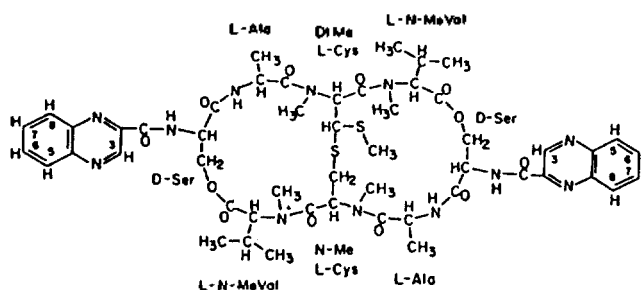


Chart I.

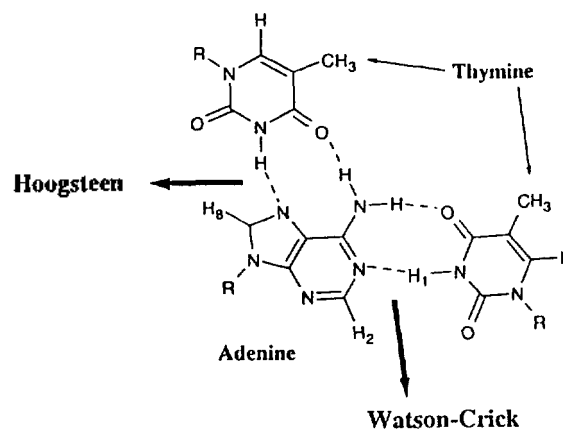
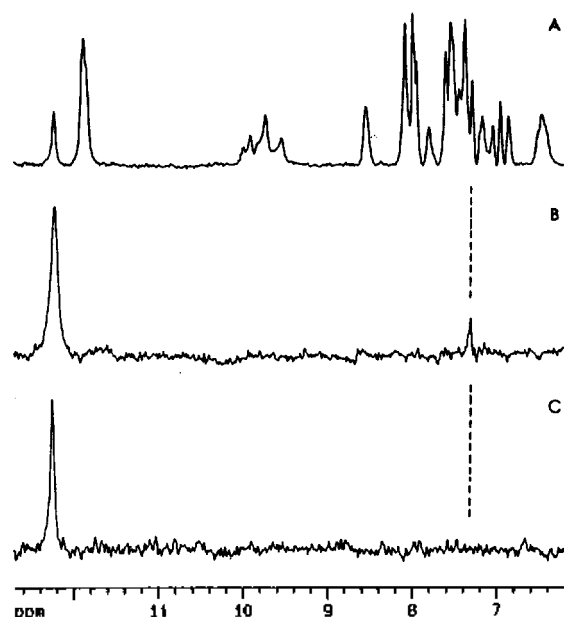


Chart II.

tube. The aqueous solution of methanol was evaporated over a period of about 12 hr under a stream of  $\text{N}_2$  gas. The sample was lyophilized and redissolved in 90%  $\text{H}_2\text{O}/10\%$   $\text{D}_2\text{O}$ . All NMR experiments were done on a Bruker AMX-500 spectrometer.

Spectrum of the imino and aromatic region of  $[d(\text{CGTACG})]_2$ -drug complex is given in Figure 1A. In the complex, all imino proton resonances were upfield-shifted by at least 1 ppm. These large upfield shifts are due to intercalative drug binding. In imino region, the lower peak at 12.22 ppm corresponds to central A · T imino proton. By comparing the  $[d(\text{CGTACG})]_2$ -drug and  $[d(\text{CGTA(D)CG})]_2$ -drug spectra, A (4) H-8 proton resonance was easily assigned to the peak at 7.28 ppm.



**Figure 1.** (A)  $^1\text{H-NMR}$  spectrum of imino and aromatic region of echinomycin- $[\text{d}(\text{CGTACG})]_2$  2:1 complex, in 0.4 ml of 0.1 M NaCl, 10 mM phosphate, pH 7 buffer, at  $1^\circ\text{C}$ . Spectrum was acquired by using the jump and return pulse sequence<sup>5</sup> ( $90^\circ$ ,  $-\Delta$ ,  $90^\circ$ ,  $-$ ) to suppress the water resonance.  $\Delta = 80 \mu\text{sec}$  was used. This sample was used for the NOE of Figure 1B. (B) NOE difference spectrum was obtained with preirradiation of central A · T imino proton resonance for 300 msec. (C) NOE difference spectrum of echinomycin complex with DNA deuterated at the adenosine H-8 position; otherwise similar to (B). Dashed lines connect resonance position of adenosine H-8 proton

NOE experiments were performed by selective saturation of A · T imino proton resonance. We were unable to observe NOE at room temperature because of rapid exchange of this imino proton with the water.<sup>2</sup> At  $1^\circ\text{C}$ , however, strong intrabasepair NOE was observed between T imino and AH8 proton resonances (Figure 1B). In the same experiment with  $[\text{d}(\text{CGTA}(\text{D})\text{CG})]_2$ -drug complex, no NOE was detected (Figure 1C). If the central A · T base pair is a standard Watson-Crick A · T base pair which has an adenosine H-2 proton adjacent to the imino proton, the NOE should remain intact in a sample of H-8 deuterated DNA-drug complex. Therefore these data clearly confirm that the observed NOE in Figure 1B originates from the short T imino-AH8 distance which is expected for Hoogsteen A · T base pairing.

In conclusion, we have demonstrated a strategy of unusual base pair identification combining NOE with specific deuteration. The method should be applicable to other unusual DNAs as well as RNAs.

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## Photoacoustic Detection of $\text{C}_{60}$

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Since the discovery of a carbon compound with a molecular weight of 720 by Smalley in 1985, a new form of solid carbon allotropes,  $\text{C}_{60}$  has been intensively studied to identify its physical properties and chemical reactivities.<sup>1-4</sup> The unique geometry, known as an icosahedral structure, and the possession of superconductivity<sup>5</sup> of this compound have stimulated a strong interest in wide research areas. Many spectroscopic properties for the Buckminsterfullerenes have been already reported in a short time through the experimental investigations such as IR,<sup>2</sup> UV-visible,<sup>2</sup> MASS,<sup>6</sup> NMR,<sup>6</sup> Raman,<sup>7</sup> and ESR,<sup>8</sup> etc. In addition, the energy level and the thermodynamic data have been obtained by the theoretical calculations.<sup>9,10</sup> In spite of such booming works, there has been little progress in the study of reactions with  $\text{C}_{60}$  due to its aromaticity which might inhibit the chemical reaction. Recently, several reports<sup>12-17</sup> on the reactions of  $\text{C}_{60}$  have opened the possibilities of further investigations regarding the reactions with the compound in many aspects of chemistry. In order to study the chemical reactions, in general, it is essential to monitor the concentration of the species participated in the reaction giving rise to the need of a simple and accurate detection for quantitative analysis. In this communication, we utilized a relatively simple photoacoustic method to detect a low level of  $\text{C}_{60}$  in the solution.

A block diagram of photoacoustic (PA) system used in this study is shown in Figure 1. The output beam of an argon ion laser (Coherent, Innova 300), operating at 351 nm, was modulated at a frequency of 29 Hz by light chopper (EG&G-9417) and was directed into the sample cell. The sample cell was made of a Borosilicate glass tube with a volume of 2 ml. An electret microphone with a built-in FET preamplifier was placed at the end of the acoustic tube. The pressure changes due to the radiation through the sample solution were detected by the microphone and amplified by a lock-in amplifier (EG&G PARC, 5210) with the time constant