[7-^{15}N]Guanosine-Labeled Oligonucleotides as Nuclear Magnetic Resonance Probes for Protein–Nucleic Acid Interaction in the Major Groove[1]

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A high degree of specificity in protein–nucleic acid interaction is thought to arise from the interaction of amino acid side chains of proteins with the polar atoms of nucleic acids in the major groove.[2] Recently, structures of a number of protein–DNA cocrystals with proteins having a helix–turn–helix or similar motif have been solved by X-ray diffraction.[3-5] These structures have basically confirmed that hydrogen bonding with major-groove polar atoms plays an important part in the determination of specificity. There is, however, no simple pattern of hydrogen bonding that allows one to enunciate a simple rule for recognition. This lack of generality in interaction patterns is demonstrated even more clearly in the case of trp repressor–operator interaction,[6] where hydrogen bonding of amino acid side chains to major-groove atoms takes place through water molecules. In addition, a number of different classes of eukaryotic DNA binding proteins have been described that do not contain the helix–turn–helix motif.[7] The mode of interaction in such proteins is unknown, although they are also throught to interact via the major groove of DNA. A study of hydrogen bonding in the major groove could thus provide us with important insights into the energetics and specificity of protein–nucleic acid interactions.

Nuclear magnetic resonance spectroscopy has been applied to a large number of structural problems of increasing complexity.[8,9] Due to severe assignment and resolution problems, selective isotope substitution with ^{13}C, ^{15}N, ^{2}H, etc. has emerged as a powerful tool in applications involving macromolecules.[10,11] In addition to powerful methods that are now available, such as isotope-directed nuclear Overhauser effects[12] to study macromolecular structure in isotope-labeled macromolecules, chemical shifts of such nuclei as ^{15}N can provide very important information on hydrogen bonding.[13,14] Labeling of major-groove nitrogen atoms, therefore, could be very useful in studying protein–nucleic acid interactions. Kupferschmitt et al.[15] have labeled the amino groups of adenine and cytosine with ^{15}N and synthetically incorporated them into oligonucleotides. Several other groups have dealt with the synthesis of ^{15}N-labeled adenine and incorporation into oligonucleotides.[16-18] N-7 atoms of purines lie in the major groove and are a major point of protein–DNA interaction. Thus labeling N-7 atoms of purine bases with ^{15}N would be particularly useful in studying protein–DNA interaction. In addition, a substantial scalar coupling constant with purine C-8H would make assignment and more sensitive indirect detection of nitrogen-15 via protons possible.

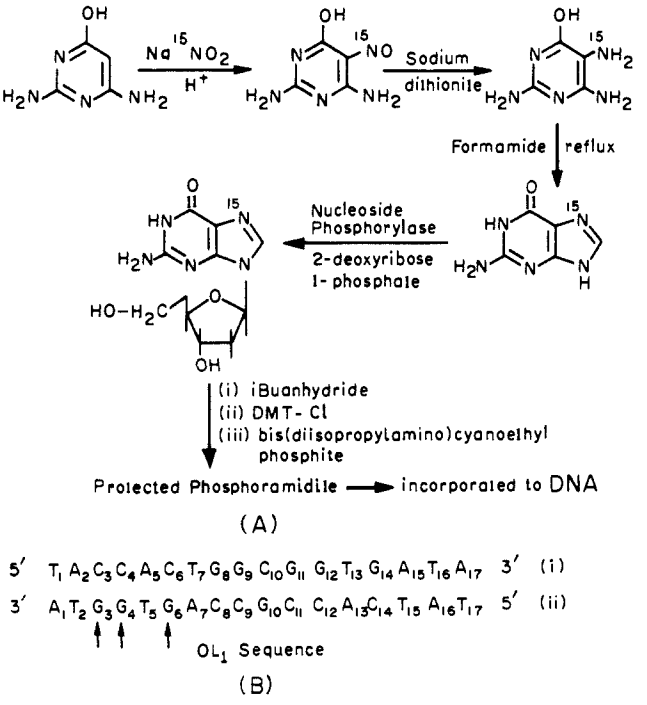

Figure 1. (A) Synthetic strategy to incorporate ^{15}N into the 7-position of guanosine and then into oligonucleotides. (B) Sequence of OL_1. Marked guanosine residues were labeled with ^{15}N.

The synthetic strategy used to incorporate ^{15}N into N-7 of guanine in a 17 base pair OL_1 operator fragment of bacteriophage λ is detailed in Figure 1. 4-Hydroxy-2,6-diaminopyrimidine was nitrosated at the 5-position by sodium nitrite and glacial acetic acid followed by reduction with sodium dithionite.[19,20] The product 4-hydroxy-2,5,6-triaminopyrimidine was cyclized with formamide, to yield [7-^{15}N]guanine.[21,22] [7-^{15}N]Guanine was then enzymatically linked with 2-deoxyribose 1-phosphate to produce [7-^{15}N]2′-deoxyguanosine.[23,24] [7-^{15}N]2′-Deoxyguanosine

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(19) In a typical preparation, 0.2 g of 4-hydroxy-2,6-diaminopyrimidine was dissolved in 10.5 mL of 10% acetic acid at 4 °C. Na^{15}NO$_2$ (0.2 g) in 2 mL of water at 4 °C was gradually added. After 2 h at 4 °C, the reaction mixture was filtered, followed by washing with cold water. The precipitate was suspended in 6 mL of water at 50 °C, and 0.6 g of sodium dithionite was gradually added. After 10 min, an additional 0.2 g of sodium dithionite was added and the reaction mixture was kept at 50 °C for 10 min and at 70 °C for an additional 10 min. On cooling, precipitate appeared, which was recrystallized from 2 N H_2SO_4.
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(21) Recrystallized and dried 4-hydroxy-2,5,6-triaminopyrimidine was refluxed in 2–3 mL of formamide for 25 min. Water (2 mL) was added, and the mixture was allowed to stand at 4 °C for 48 h and then filtered.
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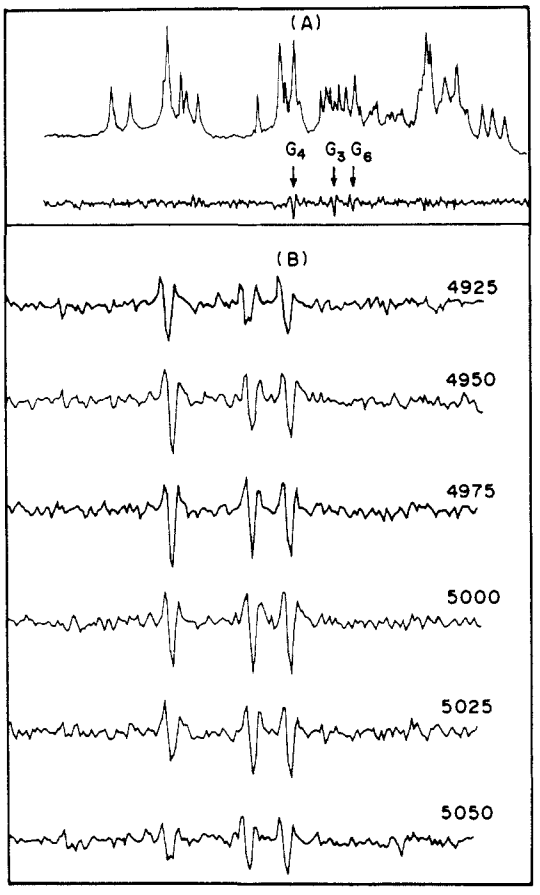

Figure 2. NMR spectra of 0.1 mM OL_1 DNA at pH 7.0 in 200 mM KCl, 50 mM potassium phosphate, 1 mM EDTA, 99.9% D_2O. All spectra were taken at 30 °C. (A) The top trace shows the aromatic proton spectrum of OL_1 in the absence of nitrogen-15 decoupling. This spectrum was the average of 2000 transients; each spectrum was accumulated at 2 Hz per point resolution, and a relaxation delay of 2 s followed each transient. The bottom trace shows the difference between spectra taken with and without broad-band ^{15}N decoupling under the same conditions as for the top trace. This trace shows that G_3, G_4, and G_6 were specifically labeled at N-7 and the protons at C-8 have chemical shifts of 7.55, 7.70, and 7.48 ppm, respectively. (B) These spectra are identical with the bottom spectrum in part A, except that they were acquired with specific-frequency ^{15}N decoupling. Each experiment was the average of 500 transients. This figure shows that the ^{15}N chemical shifts for the three guanosines are somewhat different; they are approximately 88.8, 88.3, and 88.8 ppm (relative to G N-1 of tRNA) for G_3, G_4, and G_6, respectively.

was appropriately blocked and the phosphoramidite derivative prepared.[25,26] The phosphoramidite derivative was then used in an Applied Biosystems 381A DNA synthesizer to prepare the 17 base pair OL_1 fragment. The operator fragments were then purified by reverse-phase HPLC according to Stec et al.[27] Incorporation of [7-^{15}N]-2′-deoxyguanosine was carried out only for three marked guanosine residues of strand ii.

Figure 2A shows ^{15}N difference decoupled spectra of the aromatic region of OL_1. Three major proton peaks can be seen in the difference spectra at 7.479, 7.545, and 7.695 ppm. They correspond to C-8H protons of guanosines 6, 3, and 4, respectively.[28] The ^{15}N-^1H two-bond coupling is 11 Hz. The spectrum of the aromatic region is similar to the reported OL_1 spectrum.[28]

Figure 2B shows selective ^{15}N difference decoupled spectra of the OL_1 fragment. The intensities of the three proton peaks are modulated at different nitrogen frequencies as the selective decoupling is stepped through at 25-Hz intervals in the ^{15}N dimension. We estimate that there is some chemical shift dispersion in the nitrogen dimension.

In conclusion, we have developed a synthetic procedure to label N-7 positions of 2′-deoxyguanosines and incorporated them in a 17 base pair synthetic oligomer. We have shown by selective difference decoupling that the three guanine residues differ somewhat in ^{15}N chemical shift. The 11-Hz coupling constant with the C-8H proton would allow use of HMQC techniques for nitrogen-15 assignment and indirect detection through C-8H protons. Significant sequence dependence of the ^{15}N-7 chemical shifts as well as ease of assignment and sensitive detection through protons should make [7-^{15}N]purine-labeled oligonucleotides valuable for studying protein–nucleic acid interaction.

Supplementary Material Available: Characterization of 4-hydroxy-2,5,6-triaminopyrimidine, [^{15}N]guanine, and [^{15}N]-2′-deoxyguanosine (1 page). Ordering information is given on any current masthead page.

(23) [7-^{15}N]Guanine (40 mL; 10 mM) in 1 N HCl was added to 200 mL of 0.2 M Tris and immediately back-titrated to pH 7.0. To this were added 100 mg of 2-deoxyribose 1-phosphate and 1 mg of calf thymus purine nucleoside phosphorylase (twice dialyzed against 0.1 M Tris chloride, pH 7.0; approximately 20 units). The reaction was monitored at 305 nm. When the absorbance decrease ceased (approximately 20 min), the reaction mixture was quickly frozen, lyophilized, and purified over a large Sephadex G-10 column (350 mL) equilibrated with pH 3.5 acetic acid.

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Synthesis of Novel Polyfunctional Nickel(II)–Nickel(II) Dimer $Ni_2Cl_2[C(SiMe_3)(PMe_3)]_2$ by Photolysis of the First [(Trimethylsilyl)diazomethyl]nickel(II) Complex $Ni[C(N_2)SiMe_3]Cl(PMe_3)_2$

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A growing number of transition-metal complexes have been reported, in which a diazoalkane is N-coordinated to the metal. Catalytic or photocatalytic elimination of N_2 produces carbenoid intermediates that have been used in synthesis and are thought to be key systems in homogeneous catalysis.[1] Occasionally, introduction of C-bonded diazoalkane functionality into transition-metal complexes has been achieved using $LiC(N_2)R$ or $Hg[C(N_2)R]_2$ reagents with $Os(NO)Cl(PPh_3)_3$,[2] $RhCl(PMe_3)_4$,[3] or $PdCl_2(PR_3)_2$.[4]

As the first example of a C-bonded 3d metal diazoalkane complex, we report on the synthesis of the Ni(II) compound $Ni[C(N_2)SiMe_3]Cl(PMe_3)_2$. Photoinduced elimination of N_2 gives rise to a novel Ni–Ni-bonded dinuclear compound containing two phosphorus ylide bridges as analyzed by an X-ray diffraction study.

Treatment of $(PMe_3)_2NiCl_2$ with 1 equiv of $LiC(N_2)SiMe_3$ in THF below −25 °C generates $Ni[C(N_2)SiMe_3]Cl(PMe_3)_2$ (**1**) in

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