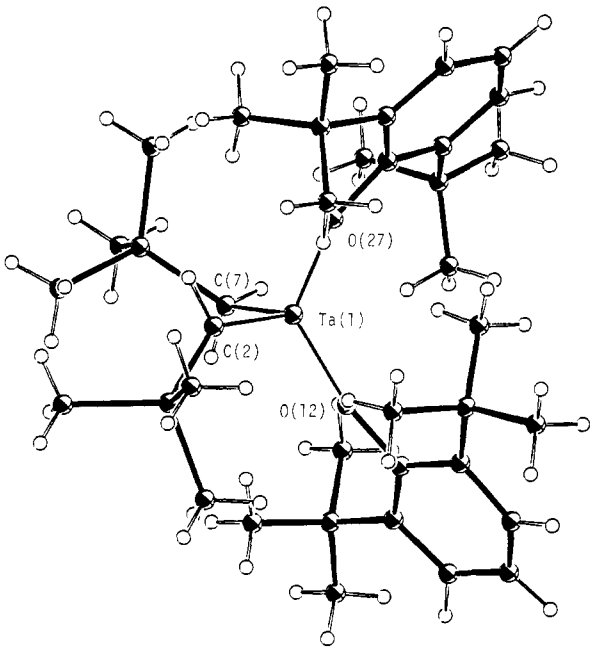

Figure 1. View of the $Ta(OC_{14}H_{21})_2(CH_2SiMe_3)(=CHSiMe_3)$ molecule. Some pertinent bond distances (Å) and angles (deg) are Ta(1)-O(12) = 1.854 (15), Ta(1)-O(27) = 1.845 (16), Ta(1)-C(2) = 1.888 (29), Ta(1)-C(7) = 2.165 (24), O(12)-Ta(1)-O(27) = 126.97 (7), O(12)-Ta(1)-C(2) = 107.4 (10), O(12)-Ta(1)-C(7) = 108.0 (8), O(27)-Ta(1)-C(2) = 108.3 (10), O(27)-Ta(1)-C(7) = 103.0 (8), c(2)-Ta(1)-C(7) = 100.1 (11).

other involves addition of the CH bond to a tantalum-carbon double bond.

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Unambiguous Assignments of the Imino Proton Resonances of a G·U Wobble Base Pair

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Guanine-uracil hydrogen-bonded base pairs, which were initially proposed by Crick in his wobble hypothesis for codon-anticodon interactions,[1] are assumed to be commonly involved in double helical structures of RNA.[2,3] Direct evidence for the presence of the G·U pair has been provided by X-ray analysis of yeast $tRNA^{phe}$ crystals.[4] ^1H NMR studies of a tRNA[5] and an rRNA fragment[3] in H_2O solution suggested that the imino proton resonances appearing in the 10-12 ppm region can be assigned to those of a G·U base pair. These assignments of G·U pair resonances of tRNA's were confirmed by nuclear Overhauser effects between the two imino protons.[6-8] However some ambiguity remains in these assignments since these tRNA's and the rRNA fragment contain many base pairs with different environments, and they can form non-Watson-Crick-type base pairs in tertiary structures. In order to define the location of the G·U pair resonances and unambiguously assign the individual signals to N^1H of G (G-N^1H) and N^3H of U (U-N^3H), we synthesized a ribotetranucleotide, G-G-C-Up, and its ^{15}N-labeled compound, G*-G-C-Up, and measured their ^1H and ^{15}N NMR spectra. When the tetramer forms a duplex, it contains two identical G·C base pairs and two identical G·U base pairs. Our results revealed that G-G-G-Up does form a duplex at low temperature, and G-N^1H of the G·C pair and U-N^3H and G-N^1H of the G·U pair give proton signals at 13.6, 12.0, and 10.6 ppm downfield from DSS reference, respectively, at 3 °C.

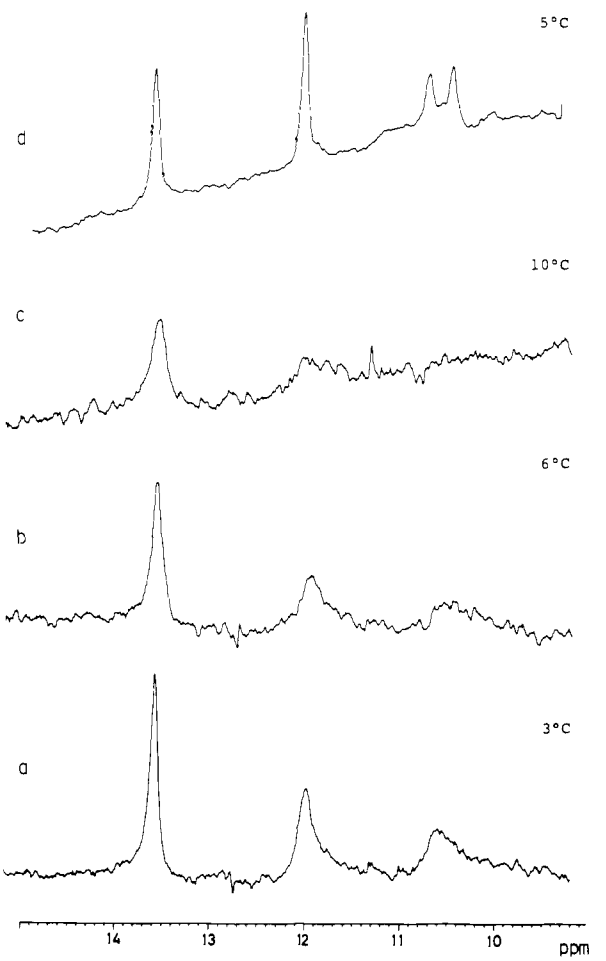

Figure 1. Imino proton region of the 360-MHz ^1H NMR spectrum in aqueous solution: G-G-C-Up (44 mM in 0.1 M NaCl), taken by Redfield pulse sequences at (a) 3 °C, (b), 6 °C and (c) 10 °C (accumulated 100 times); (d) 95% ^{15}N-enriched G*-G-C-Up (73 mM in 0.1 M NaCl), taken by normal single-pulse method with 16 bits AD converter (accumulated 100 times).

G-G-C-Up was synthesized by the modified triester method.[9] G*-G-C-Up, which contains a uniformly ^{15}N-labeled guanosine residue (95% enrichment) at the 5′-terminal, was synthesized by essentially the same procedure except that a 2′-*O*-tetrahydrofuranyl group was used for protection of the 5′-terminal unit. The low-field

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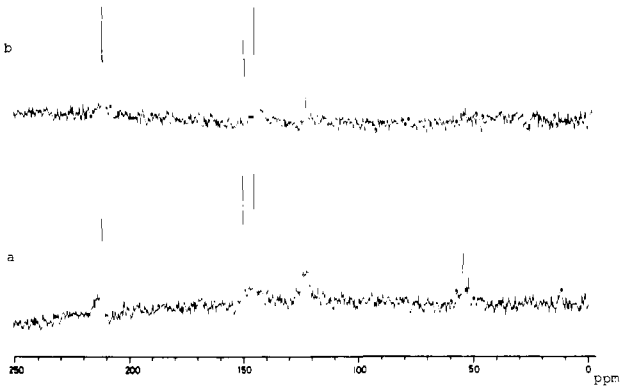

Figure 2. ^{15}N NMR spectra of 95% ^{15}N-enriched G*-G-C-Up in aqueous solution (70 mM in 0.1 M NaCl): (a) without proton decoupling (accumulated 100 times by 45° pulse with 10-s repetition interevals); (b) with selective proton decoupling (irradiated at 10.6 ppm in proton resonance frequencies and accumulated 100 times by 45° pulse with 10-s repetition intervals).

360-MHz ^1H NMR spectra of G-G-C-Up in H_2O (strand concentration is 44 mM in 0.1 M NaCl solution) were taken by Redfield pulse sequences[11] and are shown in Figure 1. At 3 °C three resonances are observed at 13.6, 12.0, and 10.6 ppm downfield from the DSS reference. Chemical shifts were initially read relative to the G-C^{8}H proton resonance and were converted to those of DSS. Raising the temperature to 10 °C causes the sharpest signal to 13.6 ppm to be still observed while the other two signals almost disappear. Therefore, this sharpest signal must be the resonance of G-N^{1}H of the G·C base pair, which is more stable than the G·U pair and, moreover, is in an internal position of the duplex. The resonance position (13.6 ppm) also supports the assignment since G-N^{1}H of G·C pair is usually observed in the 12–14 ppm region. The other two signals, which disappear simultaneously upon increasing temperature, must be G-N^{1}H and U-N^{3}H of the G·U pair. These assignments were supported by the appearance of an NOE at the 12.0 ppm peak under weak single-frequency preirradiation in 10.6 ppm. The observation pulse was applied with 1-ms delay after preirradiation for 0.1 s.[6]

The G-N^{1}H of the G·U pair was unambiguously assigned by measuring ^1H NMR spectra of G*-G-C-Up (Figure 1d). The 5′-terminal guanosine residue contains ^{15}N, and the imino proton should be coupled with the ^{15}N. When the spectra of G-G-C-Up and G*-G-C-Up are compared, the imino proton resonance of G*-G-C-Up in the highest field shows a splitting (J_{NH} = 90 Hz) and, therefore, must be the G*-N^{1}H of the G·U base pair. The relative resonance intensities of the G·U imino proton signals of G*-G-C-Up are larger than those of G-G-C-Up because of higher melting temperature due to higher concentration (70 mM) of the tetramer. In proton-coupled 36-MHz ^{15}N spectra at 5 °C, five peaks are observed at 52, 122, 144, 148, and 210 ppm downfield from external $^{15}NH_4NO_3$ (Figure 2). The first two of the above resonances are a triplet and a doublet, respectively, with 90–80-Hz coupling constants, and the latter three peaks are singlets. They could be assigned to $^{15}NH_2$, $^{15}N^1$, $^{15}N^3$, $^{15}N^9$, and $^{15}N^7$, respectively, from the coupling pattern and by comparison with previous data of ^{15}N-GMP.[12] Upon single-frequency irradiation at 10.6 ppm in the proton resonance frequencies, the $^{15}N^1$ signal becomes a singlet. Therefore the above assignment for G-N^{1}H of the G·U base pair was confirmed. The $^{15}NH_2$ resonance at 52 ppm was also found to have very low intensity. This is due to negative NOE by the saturated amino proton transferred from the irradiated imino proton.

The intrinsic resonance positions of hydrogen-bonded U-N^{3}H and G-N^{1}H in tRNA have been estimated by Geerdes and Hilbers[13] to be 12.5 ± 0.1 and 12.2 ± 0.1 ppm, respectively, by comparing the observed data and calculated ring-current chemical shifts. The estimated chemical shifts according to their calculation (12.2 and 11.7 ppm) are quite different from those observed for the present tetramer. X-ray crystallographic study of this tetramer is now being undertaken.[14]

Registry No. G-G-C-Up, 75902-87-3; G*-G-C-Up, 83731-12-8; G, 73-40-5; U, 66-22-8.

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Four-Center Cyclic Transition States and Their Associated Deuterium Kinetic Isotope Effects: Hydrogenolysis of *n*-Octyllithium[1a]

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The temperature dependence of the kinetic isotope effect provides a delicate probe of selected transition-state qualities. Thus, for example, reactions that proceed through a rate-determining step that involves a linear hydrogen transfer are recognized by Arrhenius parameters of characteristic magnitude.[2,3] However, a controversy exists concerning the characteristic values for these parameters when hydrogen transfer occurs via a significantly nonlinear transition state. Theoretical considerations[4] of hydrogen isotope effects for such transition states as well as related calculations[5] suggest that there is no basis for expecting nonlinear transition states to result in a temperature-independent isotope effect or preexponential factor much larger than $\sqrt{2}$, the theoretical upper limit for linear H transfer. However, others[6] suggest that reactions that proceed through a rate-determining nonlinear hydrogen transfer are characterized by a hydrogen isotope effect that is temperature independent and an Arrhenius preexponential factor that is significantly greater than $\sqrt{2}$. Thus, a clear dichotomy exists. In an effort to resolve this ambiguity we have examined the temperature dependence of the kinetic isotope effect associated with a transition state in which the requirement of a significantly nonlinear hydrogen transfer is reasonably unequivocal.[7]

The hydrogenolysis of *n*-octyllithium[8a] proceeds readily and quantitatively in decane solution under an ambient pressure of hydrogen and at temperatures <100 °C. The kinetics for this reaction, which is pseudo first order in *n*-octylllithium, are summarized in Table I along with the corresponding data for the equivalent reaction with deuterium. Also summarized are the relevant data obtained under competitive conditions. Within experimental error, both independent and competitive reactions exhibit equivalent temperature dependencies.

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