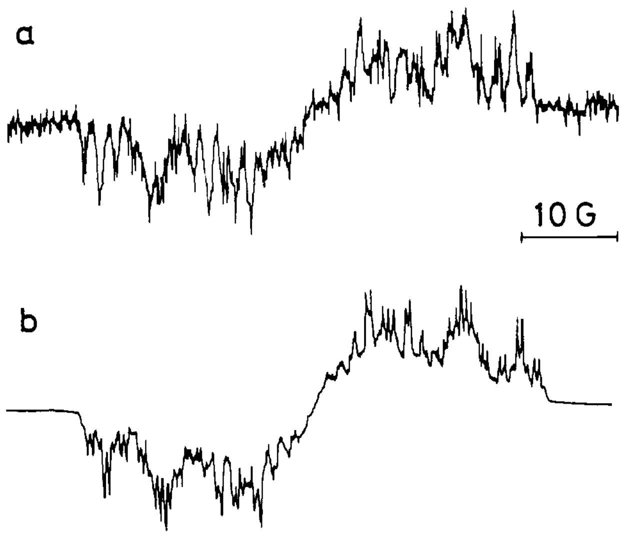

Figure 2. (a) Transient ESR spectrum observed at 1 μs after the laser pulse irradiation of the wet methanol solution containing **1** and methylviologen dichloride at −70 °C. (b) The simulated spectrum with E/A polarization. Hyperfine splitting constants for the 2-acetylbenzyl radical: 15.5 (2 H), 5.25 (1 H), 6.25 (1 H), 1.75 (2 H), and 0.50 G (3 H).

observed CIDEP can be reproduced. Since the phase of the $|\Delta M_S| = 2$ line is emissive, the most populated sublevel would be the upper one. In the present case, the E value is small, so the latter case can be neglected because of a small difference between W_x and W_y. Consequently, it may be suggested that the anisotropic ISC occurred so as to make a spin distribution $P_y > P_z >> P_x$, where P_i is the population of a triplet sublevel.

According to a conception that the biradicals formed as a result of intramolecular hydrogen abstraction in the triplet excited state of *o*-alkyl-substituted aromatic carbonyl compounds are describable as the triplets of the corresponding photoenols,[9] the transient species would be assigned to the excited triplet molecule of the enol (i.e., 1,4-biradical).[10] The presence of oxygen in solution significantly reduced the intensity of the polarization, showing a quenching of the transient species by oxygen.

The biradical is quenched by an appropriate electron acceptor to produce the monoradical as follows. Under a steady-state irradiation to the wet methanol (1 : 10, v/v) solution containing **1** (50 mM) and methylviologen (MV^{2+}, 50 mM) at −70 °C, the observed ESR spectrum was only due to the cation radical $MV^{\bullet+}$. Time-resolved ESR measurement of the same solution however exhibited the spectrum of another radical species, which showed the E/A pattern of polarization and the g value slightly different from that of $MV^{\bullet+}$ (Figure 2a). Since the photolysis of the unsubstituted acetophenone in the presence of MV^{2+} did not exhibit any polarized ESR signal in the same experimental conditions, possibility of the direct electron transfer from the ketone triplet state to the acceptor can be ignored, regardless of an appearance of the ESR signal under a steady-state irradiation. A transient ESR spectrum similar to that of Figure 2a was also obtained for the solution containing **1** and 1-benzyl-4-*tert*-butylpyridinium chloride at room temperature. From the computer simulation (Figure 2b), the generated radical was identified as 2-acetylbenzyl radical, which has been expected as one of the intermediates in the electron-transfer reaction of type II biradicals.[9] We thus concluded that the observed CIDEP spectrum originated from chemical quenching of the intermediate of photoenolization of **1** by MV^{2+} in solution. Since the polarization depends on the hyperfine splittings, the E/A polarization arises from the ST_0 mixing of the radical pair.

These results support that the observed spectra of Figure **1** are due to the intermediates in the photoenolization of *o*-methyl-substituted aromatic carbonyl compounds.

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RNA Fragment r(CGm^{5}CGCG) That Exhibits Two Conformations in Slow Exchange on the NMR Time Scale in Low Salt Solution

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Examination of the three-dimensional structure of a transfer RNA (tRNA) molecule—by far the best known among nucleic acids—shows that the polynucleotide chain undergoes many changes in direction which are associated with local conformational changes.[1] Several studies suggest that the presence of numerous atypical bases found in tRNAs—such as 5-methylcytosine (m^{5}C) or pseudouracil (Ψ)—play a specific role in the spatial organization of these molecules. For example, we showed that the substitution of uracil by pseudouracil induces a notable conformational change in an isolated ribonucleotide by favoring the syn orientation of the base;[2] moreover several works demonstrated that m^{5}C, which is also found in DNAs, favors the transition from the B to the Z form.[3-7] Recently it has been shown that the tetramer r(CGCG) is able to adopt a Z conformation in high salt solution conditions.[8]

In order to investigate the role of m^{5}C in tRNA molecules, we have examined the conformation of the self-complementary RNA fragment r(CGm^{5}CGCG) in aqueous solution by ^{1}H and ^{31}P NMR at 500 and 202 MHz, respectively.

The hexamer r(CGm^{5}CGCG) was synthesized in solution by the phosphotriester method. The purity of the hexamer was checked on a Nucleosil C18 analytical column (99.8%) and by polyacrylamide gel electrophoresis under denaturing conditions. Enzymatic digestion by snake venom phosphodiesterase indicated that r(CGm^{5}CGCG) had the expected 3′–5′ phosphodiester linkage. ^{1}H and ^{31}P NMR spectra were obtained on a Bruker WM-500 spectrometer.

Figure 1 shows the aromatic portion of the ^{1}H NMR spectra of r(CGm^{5}CGCG) in aqueous solution (0.1 M NaCl) recorded at different temperatures. The high-temperature spectrum exhibits the usual signals of the three guanosine H8 protons and of the three cytosine H6 protons. If the temperature is lowered, several base protons (Figure 1) as well as H1′ sugar proton signals (not

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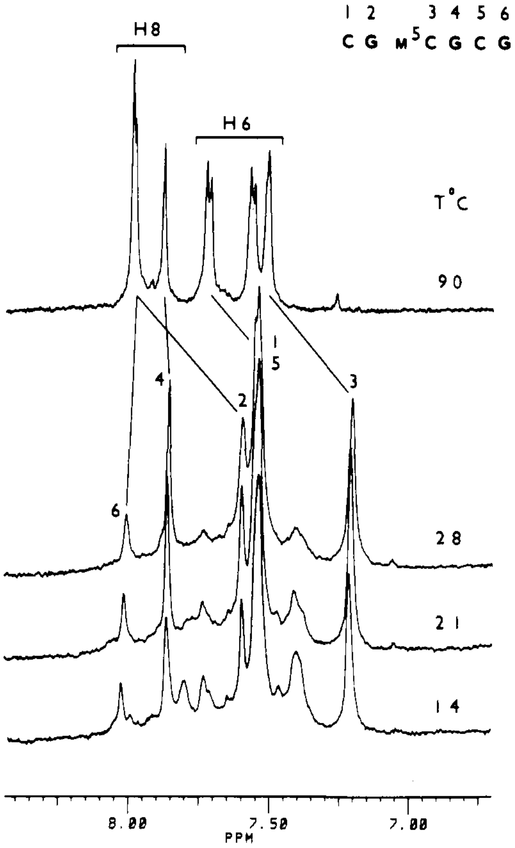

Figure 1. ^1H NMR (500-MHz) spectra (aromatic part) of r(CGm^{5}CGCG) (2 mM, pH 6.9, 0.1 M NaCl, 5 mM PO_4^{2-}) in aqueous solution recorded at different temperatures. Assignment was performed by studying two-dimensional NOE (Nuclear Overhauser effect) spectra. The weakness of the external guanine (6) H8 signal is due to H–D chemical exchange occurring at high temperature. Three overlapping resonances are included in the signal located at 7.4 ppm.

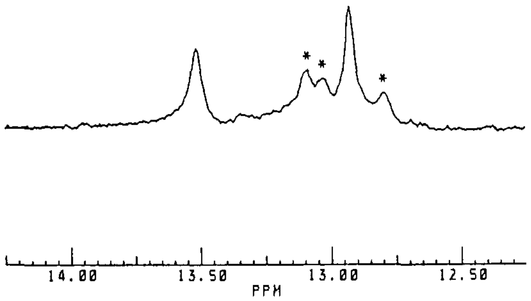

Figure 2. ^1H NMR (500-MHz) spectrum of the guanine imino resonances of r(CGm^{5}CGCG) (2 mM, pH 6.9, 0.1 M NaCl, 5 mM PO_4^{2-}) in aqueous solution (80% H_2O; 20% D_2O) recorded at 1 °C. The three imino signals of the second observed duplex are labeled with an asterisk.

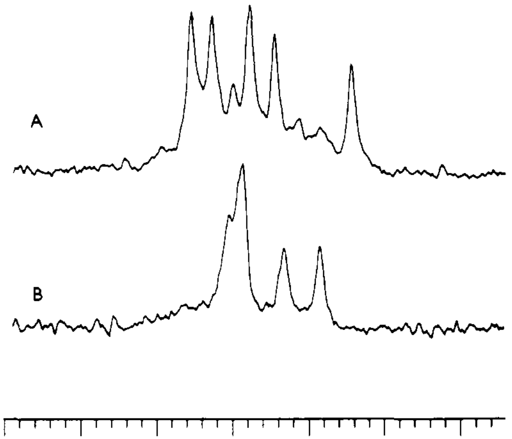

Figure 3. Proton-decoupled (202-MHz) ^{31}P NMR spectra of (A) r(CGm^{5}CGCG) and (B) r(CGUAm^{5}CG) (2 mM, pH 6.9, 0.1 M NaCl, 5 mM PO_4^{2-}) in aqueous solution recorded at 10 °C. Phosphoric acid was used as an external reference.

shown) undergo an upfield shift. The temperature dependence of the chemical shifts is sigmoidal and reflects a single strand to duplex transition; the midpoint temperature determined from the most significant shifts is about 70 °C. This value is close to that observed for the coil to A duplex transition of r(CGCGCG) (65 °C) in the same salt condition (0.1 M NaCl).[9] At 35 °C, the duplex proportion of r(CGm^{5}CGCG) is practically 100%. If the temperature is lowered below 35 °C, new resonances appear (Figure 1) whose intensity increases as the temperature decreases. In parallel, the six signals previously observed decrease while their chemical shifts are unchanged. Saturation transfer experiments between the two sets of resonances (steady-state and T_1 measurements as described in ref 7) confirm the existence of two forms in slow exchange on the NMR time scale and provide life-time values consistent with the observed line widths. Increasing the temperature up to 90 °C leads to the spectra previously observed; thus, the transition is reversible.

The two-states equilibrium observed below room temperature is also evidenced by the guanine imino proton spectrum (Figure 2): the first form exhibits two imino resonances located at 13.5 and 12.9 ppm which are in chemical exchange with three imino signals observed between 13.1 and 12.8 ppm (labeled with an asterisk in Figure 2). Our data indicate that (i) two duplexes are in equilibrium and (ii) in the first observed duplex the signal of the external guanine imino proton is broadened beyond detection whatever the temperature.

Figure 3A displays the proton-decoupled ^{31}P NMR spectrum of r(CGm^{2}CGCG) recorded at 10 °C. As expected, two sets of resonances are observed: (i) five major signals corresponding to the five phosphate groups of r(CGm^{5}CGCG) in the first observed duplex form; (ii) three minor resonances relative to the second form in slow exchange with the former. Also shown in Figure 3 is the ^{31}P spectrum of r(CGUAm^{5}CG) (in the same salt and temperature conditions) which is quite similar to that of r(CGCGCG):[9] the selfcomplementary hexamer r(CGUAm^{5}CG) adopts as r(CGCGCG) the usual A double helical form (unpublished data). Comparison of parts A and B of Figure 3 reveals that the ^{31}P resonances of the second r(CGm^{5}CGCG) duplex coincide with the A-type spectra of r(CGCGCG) and r(CGUAm^{5}CG).

Conformational analysis of the first duplex (predominant at room temperature) was performed by using scalar coupling constant measurements and two-dimensional COSY and NOESY spectra. Our data show the following: (i) the residues 2–5 adopt a C3′-endo sugar conformation as in an A-type helix[10] (J(H1′H2′) < 1 Hz, Figure 4), whereas the C(1)–H1′ signal displays a coupling constant of 8 Hz characteristic of a C2′-endo sugar ring conformation; the J(H1′-H2′) value of the G(6)–H1′ signal is 5 Hz and can be related to either an intermediate ring conformation between C3′- and C2′-endo conformation or a rapid exchange between the two forms; (ii) intranucleotide base-sugar NOEs show that all the bases exhibit a anti-type orientation; internucleotide base-sugar NOEs are consistent with an A-like conformation[9] as far as the internal residues (2–5) are concerned; in particular, H2′ sugar proton is connected via dipolar interaction to the base proton (H8/H6) of the following residue with respect to the 5′–3′ direction (H2′–H6/H8 ~2.5 Å).

Comparison of r(CGm^{5}CGCG) with r(CGCGCG) and r(CGUAm^{5}CG) A helices suggests that the presence of a 5-methylcytosine in an appropriate sequence (r(CG)$_3$) induces—

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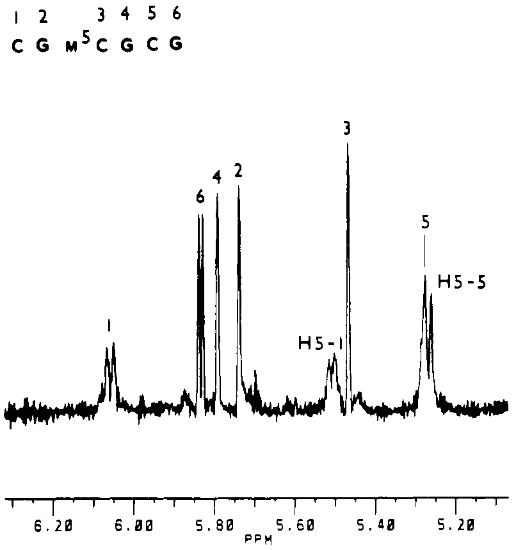

Figure 4. ^1H NMR (500-MHz) spectrum (resolution enhanced) of H1′ and H5 protons of r(CGm^{5}CGCG) (2 mM, pH 6.9, 0.1 M NaCl, 5 mM PO_4^{2-}) in aqueous solution recorded at 28 °C. Assignment was performed by studying two-dimensional NOE (Nuclear Overhauser effect) spectra. The two cytosine H5 signals are labeled H5-1 and H5-5.

probably via a hydrophobic effect—a distortion of the phosphate backbone which leads to a dramatic change in the sugar pucker of the terminal residues and weakens the corresponding hydrogen-bonded base pairing; however, the four internal residues are found in an A-like conformation. If the temperature is lowered below 28 °C, the first duplex is in slow exchange at the NMR time scale with another duplex whose phosphate backbone organization is close to that observed for r(CGCGCG) and r-(CGUAm^{5}CG).

Further information concerning the kinetics, thermodynamics, molecular process relative to r(CGm^{5}CGCG), as well as studies of other RNA sequences, will be reported in future publications.

Isolation and X-ray Crystal Structure of the Boron Methylidenide Ion $[Mes_2BCH_2]^-$ (Mes = 2,4,6-$Me_3C_6H_2$): A Boron-Carbon Double Bonded Alkene Analogue

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A recent report from this laboratory gave details of the structure of the boron-stabilized carbanion $[CH_2C_6H_2(3,5\text{-}Me_2)(4\text{-}BMes_2)]^-$, **1**,[2] which possesses a B–C bond length of 1.522 (10) Å (B–C bond order ~ 1.5). it was obtained by the deprotonation of a carbon center remote from the boron site and was stabilized by conjugation through the aromatic ring. However, there are a number of literature reports dealing with spectroscopically characterized boron-stabilized carbanions arising from deprotonation of a carbon α to the boron center.[3] The facile generation and stabilization of these interesting species are made possible through the use of bulky substituents on boron. In this regard it has been known for some time that two or more mesityl substituents can impart considerable kinetic stability toward reactions at a boron center.[4] This fact led to the isolation and characterization by X-ray crystallography of the stable radical anion $BMes_3^{\bullet-}$[5] and the ion **1**.[2] Additionally, Wilson,[6] Pelter,[7] and co-workers have shown that the in situ deprotonation of Mes_2BMe, **2**, with LiMes or $LiN(C_6H_{11})_2$ affords solutions of the anion $[CH_2BMes_2]^-$, **3**, and this anion displays many of the characteristics of ylides.[7] We now report the structure of this anion and show that it has a short B–C bond length of ca. 1.44 Å consistent with a boron–carbon double bond formulation.

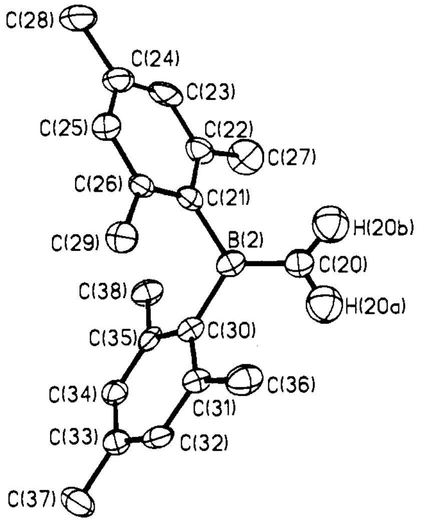

Figure 1. Computer-generated drawing of an $[Mes_2BCH_2]^-$ anion. Important bond distances (Å) and angles (deg), with values for the other crystallographically independent ion in brackets, are as follows: B(2)C-(20) = 1.438 (9) {1.450 (7)}, B(2)C(21) = 1.618 (7) {1.627 (7)}, B(2)-C(30) = 1.614 (7) {1.610 (8)}, C(20)H(20a) = 1.00 (5), C(20)H(20b) = 1.05 (6) {C(1)H(1a) = 1.04 (5), C(1)H(16) = 0.95 (5)}, C(20)B(2)-C(21) = 122.0 (4) {121.0 (5)}, C(20)B(2)C(30) = 121.6 (4) {120.7 (5)}, C(21)B(2)C(30) = 116.4 (5) {118.3 (4)}, H(20a)C(20)H(20b) = 112 (5) {112 (4)}, H(20a)C(20)B(2) = 121 (4) {122 (3)}, H(20b)C(20)B(2) = 128 (6) {126 (3)}. Dihedral angles of the rings C(21) and C(30) with respect to the central core are 53.3 {56.1} and 59.2 {52.9}.

The title anion **3** can be crystallized in ca. 40% yield as colorless parallelepipeds (mp 112–114 °C) when 2 equiv of 12-crown-4 are added to the THF solution of its lithium salt.[5,8] The latter may be generated by the literature methods.[6,7] The structure of **3** is illustrated in Figure 1.[9] The compound crystallizes as two crystallographically independent but chemically equivalent ion pairs. The most notable feature of the molecule is the short B–CH_2 bond, which has the values 1.438 (9) and 1.450 (7) Å for an

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(9) Crystal data for $[Li(12\text{-crown-}4)_2][Mes_2BCH_2]\cdot 0.75THF$ at 130 K with Mo Kα radiation: $a = 46.251$ (24) Å, $b = 15.290$ (6) Å, $c = 23.816$ (13) Å, $\beta = 114.70$ (3)°, monoclinic, space group $C2/c$, $Z = 16$, 7279 unique reflections with $I > 2\sigma(I)$, $R = 0.082$.

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