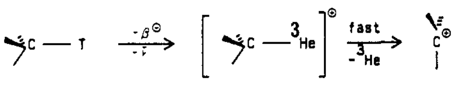
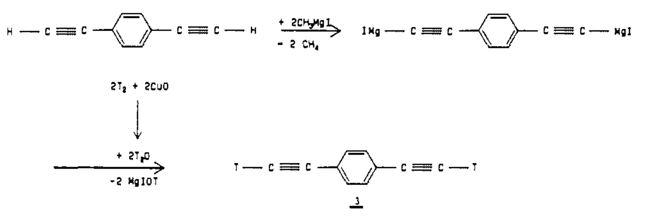
Scheme I

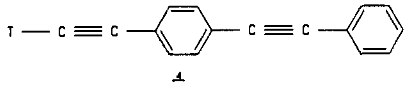


Scheme II

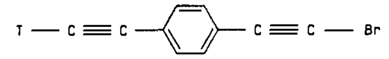


allowing investigation of their reactivity in the gaseous and condensed phase. This nuclear decay procedure has been extended to the generation of the alkynyl cation **1**. Thus 1,4-bis(tritioethynyl)benzene (**3**) was synthesized according to the reaction sequence given in Scheme II.[11] The synthesis was carried out in very dry sealed reaction vessels. The product from blank deuteriation experiments has been examined by MS and ^{1}H NMR analyses. 1,4-Bis(deuterioethynyl)benzene was formed in these blank runs in over 90% yield. Radio HPLC and GC of the tritiated reaction mixture showed the exclusive formation of **3** in comparable yields.[12] The mixture, extracted with inactive benzene, was subjected to azeotropic distillation to remove residual tritiated water. Samples of the purified solution corresponding to ca. 1 mCi (18.7 Ci mol^{-1}) of **3** were introduced into Pyrex ampoules containing variable amounts of liquid or gaseous nucleophiles. After sealing they were stored in the dark for ca. 6 months at room temperature. To avoid selective loss of the acidic tritium atoms of **3** by prototropic exchange with the nucleophile during the storage period, only aprotic nucleophiles (benzene, 1,4-dibromobutane, *tert*-butyl bromide) were employed to trap the ionic species arising from the nuclear decay of **3**.

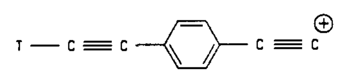
By using authentic samples for comparison, radiochromatographic analysis of the decayed mixtures revealed the formation of significant yields of **4** (96%) from liquid benzene (0.3 mmol) and of **5** (36 and 31%) from liquid 1,4-dibromobutane (8.3 mmol) and *tert*-butyl bromide (8.9 mmol), respectively. The absolute yield of **5** decreased to 7% and 9% in gaseous *tert*-butyl bromide at 10 (0.26 mmol) and 50 Torr (1.33 mmol), respectively. Most of the residual activity recovered in the mixture containing brominated nucleophiles is due to polybrominated products, which are under investigation. Formation of the tritiated products is not accompanied by any detectable amounts of the corresponding unlabeled forms (<10^{-5} mol%). This excludes any other conceivable (thermal, photochemical, radiolytic, etc.) source for the radioactive products **4** and **5**.



4



5



6

Isolation of tritiated products **4** and **5** from the corresponding decay mixtures could point to the actual occurrence of the tritiated alkynyl cation **6** from spontaneous nuclear decay in **3** and its subsequent trapping with a nucleophile.

The same products may well arise from displacement of the ^{3}He in the primary daughter ion $[TC{\equiv}C{-}C_6H_4{-}C{\equiv}C{-}^3He]^+$ by the nucleophile. In fact, at variance with the repulsive nature of most common C–He bonds,[13] the C–He dissociation in $[H{-}C{\equiv}C{-}He]^+$ has been calculated to range about 1 eV.[14] However, this energy can be readily provided by the recoil energy (maximum value of several eV) imparted to the ^{3}He moiety by the leaving β^- and $\bar{\nu}$ particles after the decay event,[15] so that a significant fraction of the primary species $[R{-}C{\equiv}C{-}^3He]^+$ is expected to dissociate rapidly to **1** before interacting with the nucleophile.

The present results provide first evidence on the transient existence of an alkynyl cation, such as **6**, in both gas phase and solution and on its behavior toward several organic molecules. The exceptional leaving group ability of the ^{3}He atom in $[R{-}C{\equiv}C{-}^3He]^+$, formed from β^- decay of $R{-}C{\equiv}C{-}T$, allows unimolecular cleavage of the C–^{3}He bond, and, therefore, formation of **1** becomes energetically accessible.

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A Rigid and Nonperturbing Probe for Duplex DNA Motion

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The structural heterogeneity of duplex DNA and its complexes with proteins suggests that not only is the *conformation* of a sequence of bases in DNA critical to normal function but also the *dynamics* of certain sequences may be important.[1] While progress has been made in the study of DNA local dynamics, experimental studies[2] of particular base sequences (other than homopolymers) have to date been prevented by the lack of a technique which focuses on a specific location in a given sequence. Electron paramagnetic resonance (EPR) spectroscopy should be well suited to detecting sequence dependent DNA dynamics[3-5] but requires a method for the site-specific introduction of a paramagnetic probe. Such studies are further complicated by the

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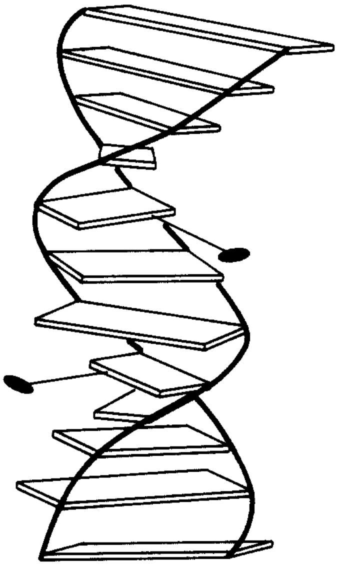
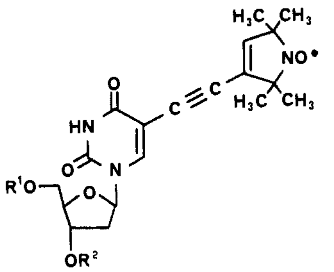


Figure 1. Schematic of the acetylene-tethered nitroxide spin label **1** after incorporation into a self-complementary dodecamer.

need to assure insignificant perturbation of the DNA as well as correlation of the motions of the spin label and DNA. We have prepared a site specifically spin labeled duplex DNA dodecamer which appears not to be structurally perturbed and provides a rotational correlation time measured by EPR which implies that the spin probe's motion is highly correlated with the tumbling of the whole macromolecule.

The phosphoramidite **1a** was synthesized[6] and incorporated[8] into the self complementary labeled dodecamer 5'-d-(CGCGAATT*CGCG), where T* represents residue **1**. Space



1

a R^1 = DMTr, R^2 = $P[N(i\text{-}Pr)_2]OCH_2CH_2CN$

b R^1 = H R^2 = PO_3^{2-}

c R^1 = H, R^2 = H

filling models indicate that this sequence is capable of adopting the B-conformation, the two spin labels being well separated (i.e., ~25 Å apart) and occupying the major grove (Figure 1). As a spectroscopic benchmark for unperturbed B-DNA, the unlabeled sequence 5'-d(CGCGAATTCGCG)[10] was also prepared.

Spectroscopic comparison of the spin labeled and unlabeled dodecamers indicates that the two are of closely related secondary structure. The CD spectra of the labeled and unlabeled sequences (Figure 2A) are quite similar to one another and are consistent with right-handed helices. Any major distortion due to the spin label would be expected to drastically alter the thermal denaturation profile;[11] the observed profiles (Figure 2B) show only subtle differences. The ^{1}H NMR data for the labeled dodecamer (Table I) provide especially strong support for a duplex as the predominant species. The assignments of the labeled duplex signals are based upon the previously reported chemical shifts of the unlabeled duplex[12] and are consistent with the degree of paramagnetic line

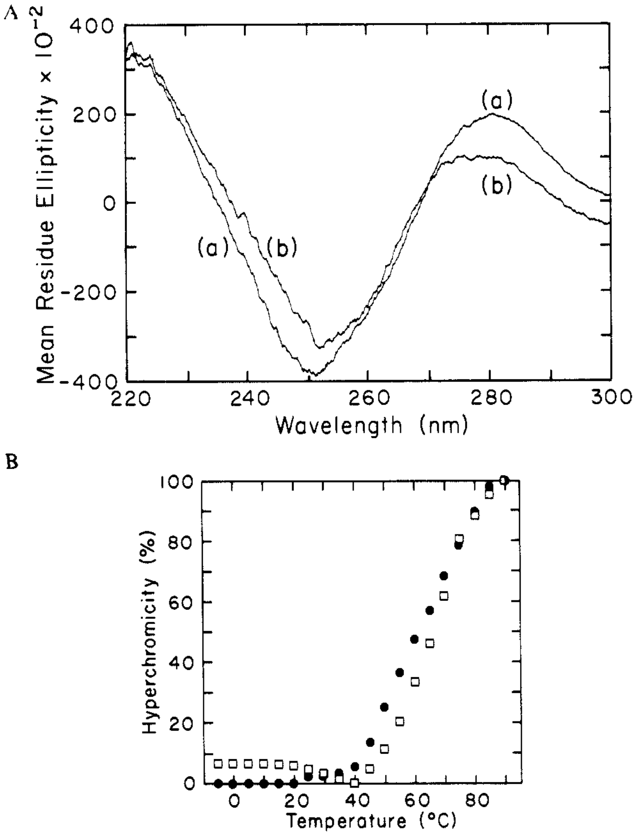


Figure 2. (A) CD spectra of 5'-d(CGCGAATTCGCG) (a) and 5'-d-(CGCGAATT*CGCG) (b) (ca. 30 μM in duplex DNA, 100 mM NaCl, 10 mM phosphate buffer, pH 7.0, 0.1 mM EDTA, 25 °C). (B) Thermal denaturation of 5'-d(CGCGAATT*CGCG) (□) and 5'-d-(CGCGAATTCGCG) (●) monitored at 260 nm (ca. 2 μM in duplex DNA, 100 mM NaCl, 10 mM EDTA). The significance of the subtle differences between the labeled and unlabeled duplexes are not at this time known.

Table I. Partial ^{1}H NMR Spectral Data for Spin Labeled and Unlabeled Duplex DNA[a]

```
     1 2 3 4 5 6 6 5 4 3 2 1
                   (*)
(5') C G C G A A T T C G C G
     | | | | | | | | | | | |
     G C G C T T A A G C G C (5')
         (*)
```

	proton				
	2	3	4	5	6
spin labeled duplex[b]					
chemical shift (δ)	12.96	12.78	12.51	14.42[c]	13.64
line width (Hz)	36	42	65	116	120
unlabeled duplex[d]					
chemical shift (δ)	12.95	12.78	12.58	13.67	13.55

[a] 500 MHz, 10% D_2O in H_2O,[17] ca. 0.25 mM, in duplex DNA, 100 mM NaCl, 10 mM phosphate buffer, pH 7.0, 0.1 mM EDTA, 25 °C. [b] Line widths of all iminos after reduction of the nitroxide with $Na_2S_2O_4$ were 22 Hz. [c] Relative to thymidine, a 0.5 ppm downfield shift was observed for the NH of **1c**, which compares favorably with the 0.8 ppm downfield shift observed for proton 5 in the labeled relative to the unlabeled duplex. [d] Line widths for all imino protons were 11 Hz.

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(6) Prepared by coupling[7] of 2'-deoxy-3',5'-di-*p*-toluoyl-5-iodouridine and 3-ethynyl-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy free radical followed by deprotection and refunctionalization of the 3' and 5' hydroxyls.

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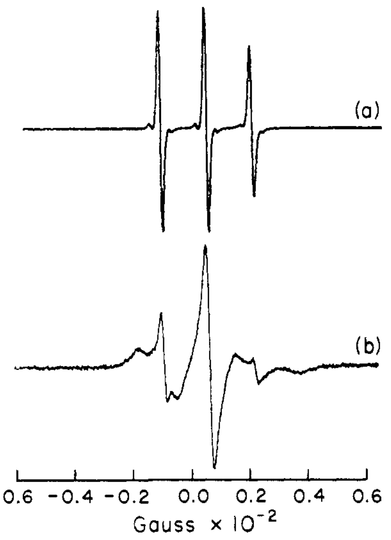

Figure 3. EPR spectra of (a) **1b** and (b) 5'-d(CGCGAATT*CGCG) (100 μM in duplex DNA, 100 mM NaCl, 10 mM phosphate, pH 7.0, 0.1 mM EDTA, 0 °C). In spectrum (b), the origin of the signal representing $\tau_r < 1$ ns (ca. 15%) which is superimposed upon the $\tau_r \approx 5$ ns signal is under investigation.

broadening[13] [T(5),T(6) > G(4) > G(3) > G(2)] which is anticipated based on the distance of these iminos from the two spin labels (see Figure 1). Reduction of the spin label (excess sodium dithionite) eliminates this differential line broadening effect.

On the basis of simple hydrodynamic considerations,[14] anisotropic tumbling in the 4–7 ns range is expected for an aqueous dodecamer duplex at 0 °C. The EPR spectra of **1b** and the spin labeled dodecamer are shown in Figure 3. As calculated from these spectra,[15] **1b** has a rotational correlation time (τ_r) shorter than 0.3 ns; in contrast, the labeled dodecamer has a τ_r of roughly 5 ns at 0 °C. That this τ_r is calculated from the EPR data without any correction for subnanosecond internal dynamics of the probe independent of the DNA[5] is a strong indication that the motion of the spin probe is well correlated with the motion of the DNA.

These studies unequivocally demonstrate (1) that the nitroxide moiety is compatible with phosphoramidite-mediated DNA synthesis, (2) that attachment of a bulky and hydrophobic nitroxide can be accommodated without disruption of DNA secondary structure,[16] and (3) that a nondisruptive spin probe can be sufficiently rigidly constrained to correlate its motion with that of the DNA to which it is attached. These findings provide a foundation for further DNA local dynamics studies in which this probe is incorporated into base sequences of biological relevance in much larger duplex DNAs. This probe should likewise find use in dynamics studies of other unusual DNA structures (i.e., hairpins, mismatches, bulges) and may have utility as a paramagnetic marker in structural studies of DNA by NMR. These studies are in progress, and the results will be reported in due course.

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Electronic Absorptions in the High T_c Superconductor $YBa_2Cu_3O_x$

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Superconductivity in metals and alloys has been well-explained by the theory of Bardeen, Cooper, and Schrieffer (BCS)[1] in which electrons are paired by a phonon-mediated, attractive interaction. The theory predicts the correct order of magnitude of the transition temperature, T_c, and a dependence of T_c on the isotopic mass of the metal. The recently-discovered ceramic superconductor,[2] $YBa_2Cu_3O_x$ with a T_c of 93 K and the virtual absence of an isotopic effect,[3,4] suggests the presence here of a different pairing mechanism. That an electronic *excit*ation (the *exciton* interaction) could provide such an interaction and give very high T_c's had been suggested previously.[5] It should be noted that the term "exciton" in the context of superconductivity does not refer to Frenkel or Wannier excitons. A strong electronic absorption has, in fact, been seen in reflectivity studies of polycrystalline samples of $YBa_2Cu_3O_x$[6] and the related compound $La_{2-x}Sr_xCuO_4$[7-9] at 0.37 and 0.5 eV, respectively. For $La_{2-x}Sr_xCuO_4$ the absorption strength varies with x in the same manner as does T_c. This suggested that an exciton interaction might be present. However, Bozovic et al.[10] found no such absorption peak in high quality, oriented films of $YBa_2Cu_3O_x$ and suggested that it was due to an impurity or surface artifact not related to superconductivity and thus did not provide evidence of an exciton interaction. Here we report spectroscopic studies of high-purity samples of $YBa_2Cu_3O_x$ as well as several impurity phases that we have identified as possible contaminants. We show that this low-lying electronic transition is characteristic of $YBa_2Cu_3O_x$, is not due to an impurity phase, and does correlate with T_c and then discuss its possible connection to an exciton interaction.

Sintered pellets of fully-oxygenated $YBa_2Cu_3O_7$ samples were prepared by standard ceramic techniques.[11,12] Oxygen-deficient samples were prepared by heating the fully-oxygenated sample under argon at elevated temperatures (500 °C for $x = 6.6$; 570 °C for $x = 6.4$; 700 °C for $x = 6.2$; 800 °C for $x = 6.0$) until the desired oxygen content was achieved and then slowly cooling to room temperature in the inert atmosphere. The oxygen content of these samples was measured gravimetrically. X-ray powder diffraction studies were performed on a Rigaku XRD instrument

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