(i) Irrespective of their source, metastable decomposition of ions II into NO_2^+ and H_2O occurs with a large release of kinetic energy (747 ± 10 meV), giving a typical, dish-topped peak (Figure 2A), while ions I decompose giving an non-Gaussian peak with a much lower kinetic energy release (Figure 2B). Such a difference is particularly significant, in that kinetic energy release is primarily dependent on the reverse activation energy not on the internal energy of the decomposing ions.[9] Large releases are typical of rearrangements, while simple bond cleavages have little or no reverse activation energies.[10] It follows that II must undergo rearrangement prior to metastable H_2O loss, which is not the case of I.

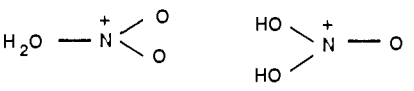
(ii) The collisional-induced dissociation (CID) spectra, depurated from the unimolecular contribution, are also largely different, the CID spectrum of I displaying three fragments, i.e., H_2O^+, NO^+, and NO_2^+, in the approximate 0.7:1:9 ratio, while that of II displays only NO^+ and NO_2^+ in the approximate 1:2 ratio.

(iii) Further structural discrimination is provided by the different metastable decomposition of the ions from the reaction

$$H_2NO_3^+ + H_2{}^{18}O \rightarrow H_2NO_2{}^{18}O^+ + H_2O \qquad (3)$$

unequivocally established by FT-ICR spectrometry and occurring as well under CI conditions.[11] Ions I from (3) undergo metastable decomposition into *unlabeled* NO_2^+, losing exclusively $H_2{}^{18}O$, while labeled ions II undergo nearly statistical (typically 2.5:1) metastable loss of H_2O and of $H_2{}^{18}O$.[12]

Overall, the above results suggest that I is the more stable isomer, characterized by a hydrated nitronium ion structure, whose metastable loss of water involves a simple bond cleavage, without a large release of kinetic energy. The presence of a discrete H_2O



I II

moiety accords well with the selective loss of $H_2{}^{18}O$ in the metastable decomposition of ions I from process (3), that amounts, in this case, to a simple ligand exchange. The features of ions II are consistent instead with a structure containing two OH groups, whose metastable decomposition into NO_2^+ and H_2O presupposes molecular reorganization, which justifies the large release of kinetic energy. The mixed isotopic composition of water from the metastable decomposition of ^{18}O-labeled ions II accords well with a structure containing no O atom in a preexistent H–O–H group, whose loss can occur preferentially, as from I, via a simple bond cleavage, requiring no preliminary rearrangement.

Our conclusions qualitatively agree with the results of SCF calculations, which identify a H_2O–NO_2^+ structure as the most stable among the isomers investigated, showing that the relative energies of ions akin to II are higher by at least 8 kcal mol^{-1}.[6]

It should lastly be noted that the kinetic energy release from II sets a lower limit of the order of 20 kcal mol^{-1} to the activation energy for the hydration of NO_2^+ yielding II and that there are reasons to believe that the free energy of activation for the II → I isomerization is correspondingly large. Remarkably, the process does not appear to be catalyzed by interaction of II with a water molecule.

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(11) Even under CI conditions, $H_2NO_2{}^{18}O^+$ ions arise exclusively from exchange 3, since no appreciable hydration of NO_2^+ has been detected in specific control experiments.
(12) The MIKE and CID spectra were recorded by using a ZAB-2F instrument (Micromass, Ltd.), operating the CI source at 160 °C. The spectra represent an average of 100 scans, with an energy resolution of 1.2 eV main-beam width.

Measurement of ^1H–^1H Coupling Constants in Oligonucleotides by 2D NMR: Application of ω_1-Decoupling

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Three-bond ^1H–^1H coupling constants constitute the basic input for the determination of conformation of d-ribose rings in oligonucleotides by NMR spectroscopy.[1] Different techniques, such as ω_1-scaled COSY,[2,3] P.E.COSY,[4] and DISCO,[5] to name a few, have been used to derive this structural information.[6-9] While all these have proved extremely useful, any procedure that enhances resolution in the spectra is always a welcome addition. In this context ω_1-decoupling in correlated spectroscopy[10,11] is an attractive proposition since this allows complete elimination of multiplicity along the ω_1-axis of the two-dimensional spectrum. In this communication we use the following experimental scheme.[11]

$$90 - (\Delta + t_1)/2 - 180 - (\Delta - t_1)/2 - 90 - \text{acquire}(t_2)$$

t_1 and t_2 are the usual evolution and detection periods, and Δ is a constant time period. In this scheme the heteronuclear H–P coupling will however be retained along the ω_1-axis of the two-dimensional spectrum. The cross peaks can be phased to pure absorption along both axes, while diagonal peaks will have disperse character along ω_2 and absorptive character along ω_1.

Figure 1 shows the (H1′)–(H2′, H2″) cross peak region of the ω_1-decoupled COSY spectrum of the oligonucleotide hairpin d(C-G-C-G-A-G-T-T-G-T-C-G-C-G). It is seen that all the expected cross peaks are present. Both positive (+) and negative (−) signals have been plotted, and the multiplet pattern along ω_2 is + − + − for H1′–H2″ cross peaks and + + − − for H1′–H2′ cross peaks. In the case of nucleotide units G2, G4, G6, G9, and G12, strong coupling artefacts are seen between the H1′–H2′ and H1′–H2″ cross peaks. In Figure 2 a particular horizontal slice through the spectrum in Figure 1 is shown in order to bring out the characteristics of the spectrum. The central + and − signals have lower intensities compared to the two outer signals; the intensity difference being more pronounced in G6. This is a consequence of (i) partial cancellation of the + − intensiteis in the center and (ii) strong coupling effects in G6 because of which the central two lines have lower intensities inherently. All the cross peak multiplets have been simulated (illustrated on the side in Figure 1) to obtain true peak positions which directly yielded the H1′–H2′ and H1′–H2″ coupling constants in all units except where strong coupling effects were observed. In the strongly coupled cases, the simulated peak positions do not yield directly the coupling constant values.

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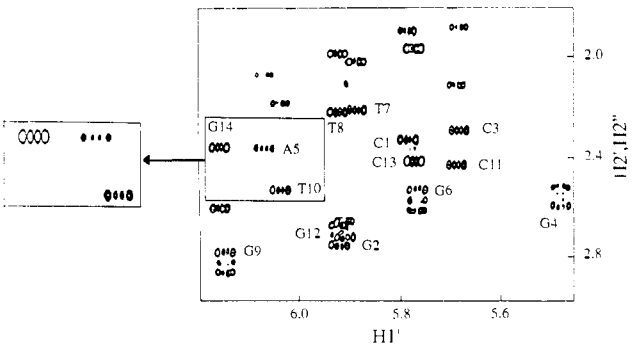

Figure 1. (H1′)–(H2′–H2″) cross peak region of the phase sensitive ω_1-decoupled COSY spectrum of the oligonucleotide d(C-G-C-G-A-G-T-T-G-T-C-G-C-G) recorded on a Bruker AM 500 FT-NMR spectrometer. Four hundred t_1 increments were used in TPPI[14] (time proportional phase incrementation) fashion, with 2048 data points (t_2) for each t_1 value. The value of Δ which corresponds to t_1^{max} here was 56 ms. The data were zero-filled to 8192 along t_2 and 2048 along t_1, prior to window multiplication by sine bell shifted by $\pi/4$ and subsequent Fourier transformation. A separate one-dimensional spectrum was used to obtain the phase constants for phase correction to pure absorption (cross peak) along the ω_2 axis of the 2D spectrum. After the 2D transform, phase correction was applied along ω_1 (taking a cross section from the spectrum) to obtain pure absorptive phases. Digital resolution along ω_2 is 0.87 Hz/point, and along ω_1, 3.48 Hz/point. Experimental time was 14 h. Only the H1′–H2″ cross peaks are labeled in the figure. Side panel shows illustrative simulations of the multiplet patterns of the H1′–H2″ cross peaks of G14, A5, and T10 units.

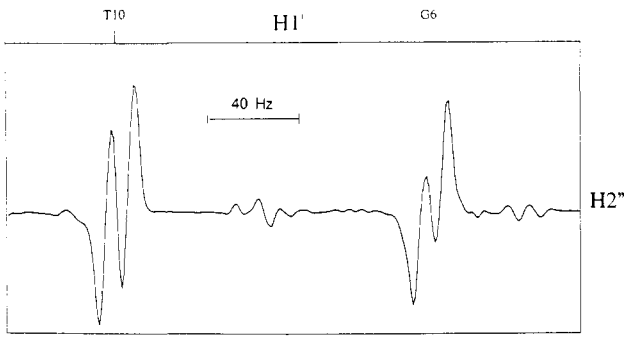

Figure 2. A horizontal cross section through the spectrum in Figure 1 showing the H1′ multiplets of T10 and G6 nucleotide units.

Figure 3 shows the (H2′, H2″)–H3′ cross peak region of the same ω_1-decoupled COSY spectrum as in Figure 1. Each peak here has the H2′ multiplicity with the arrangement of +, − signals as (+ − + + − − + −). These multiplet patterns have also been simulated (illustrated on the top of the figure) to obtain the H2′–H3′ coupling constant in the units A5, T7, T8, T10, C11, and G14. C1 and C3 peaks overlap significantly and are not amenable to proper simulation. For the G2, G4, G6, G9, and G12 units, the H2′–H3′ coupling constant could not be obtained accurately owing to strong coupling complications. The C13 peak has been bleached out by water irradiation. H2″–H3′ cross peaks are extremely weak in every case, which must be attributed to the very small (1–1.5 Hz) value of the H2″–H3′ coupling constant. These cross peaks are not seen in any of the J-correlated spectra of the oligonucleotide. The same conclusion holds good for the H3′–H4′ coupling constant.

Thus it seems possible that most of the measurable coupling constants can be obtained from one ω_1-decoupled COSY spectrum, and the coupling constants measured in the present case are listed in Table I. The data can be analyzed for sugar geometries by following the functional dependence of the individual coupling constants on sugar geometries.[12,13] Suffice it to say that the data are consistent with sugar geometries in a narrow domain around

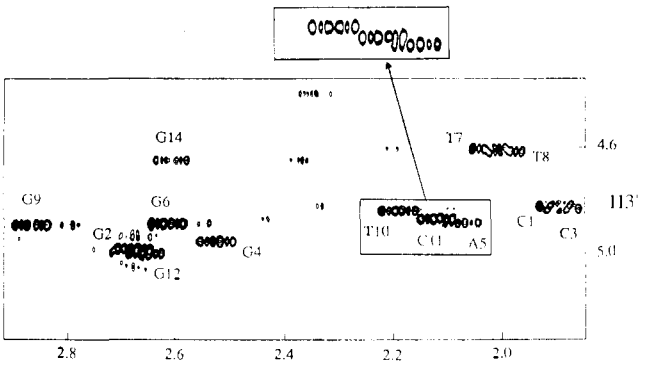

Figure 3. (H2′–H2″)–(H3′) cross peak region of the same ω_1-decoupled COSY spectrum as in Figure 1. All the cross peaks are seen except for C13 which was bleached out by irradiation. On the top illustrative simulations of H2′–H3′ cross peaks of T10, C11, and A5 units are shown.

Table I. Measured ^{1}H–^{1}H Coupling Constants from the ω_1-Decoupled COSY Spectrum of d(C-G-C-G-A-G-T-T-G-T-C-G-C-G)

	J (H1′–H2″)	J (H1′–H2′)	J (H2′–H3′)	J (H2′–H2″)
C1	5.7	9.6		
C3	5.2	9.2		
C13	5.7	9.8		
A5	5.9	10.4	5.2	−14.0
T7	5.2	10.0	5.4	−14.0
T8	5.0	9.8	6.1	−14.0
T10	5.0	9.1	6.3	−14.0
C11	5.2	9.2	6.2	−14.0
G14	5.0	9.8	5.1	−14.0

C2′-endo conformation for all the measured nucleotide units.

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p-Diphenoquinone Analogues Extended by Dihydrothiophenediylidene Insertion: A Novel Amphoteric Multistage Redox System

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Organic molecules having an enhanced amphoteric redox property,[1] namely, a small span of the oxidation and reduction potentials $E^{sum} = E^{ox} + (-E^{red})$ which is oxidized and reduced easily by a multistage electron transfer, have attracted much attention in recent years in relation to single-component molecular assemblies with electrical conductivity.[2] The most definitive example would be graphite exhibiting $E^{sum} = 0$. In order to build up a novel closed-shell system with an amphoteric multistage redox property, we designed extended quinones in which one or more dihydrothiophenediylidene moieties are inserted between the two

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