bonding network in both the proximal and distal pocket. These studies will be reported in the near future.

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Communications to the Editor

Measurement of ^1H–^{31}P NMR Coupling Constants in Double-Stranded DNA Fragments

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NMR presents a unique tool for obtaining detailed conformational information about DNA fragments in solution. So far, most studies have relied on measurements of NOE buildup rates and of ^1H–^1H coupling constants.[1] Here, a new method is proposed that permits measurement of previously unresolvable $^3J_{HCOP}$ couplings, providing additional structural information about the DNA backbone. A Karplus relationship correlating $^3J_{HCOP}$ with the H–C–O–P dihedral angle has been proposed by Lankhorst et al.:[2]

$$J_{HCOP} = 15.3 \cos^2 \phi - 6.1 \cos \phi + 1.6 \qquad (1)$$

Because of the complexity of both the ^{31}P and C3′H, C5′H′, and C5′H″ multiplet structures, measurements of the J_{HP} couplings in DNA have been limited to small fragments with very narrow line widths. It is demonstrated here that for larger fragments it is also possible to measure the J_{HCOP} couplings, provided all other multiplet splittings are suppressed.

Homonuclear couplings to the C3′H resonance can be removed in a 2D experiment by the application of a selective 180° refocusing pulse in the middle of the evolution period, affecting only the C3′ protons. Since the C3′H resonances in DNA usually are separated from the C4′ and C2′ protons, such an experiment presents no particular difficulty. Two possible schemes incorporating this idea are

$$\begin{array}{ll} ^1H: & 90_x\text{–}t_1/2\text{–}90_{\phi,sel}\text{–} \quad 180° \quad \text{–}90_{\phi,sel}\text{–}t_1/2\text{–acq } (\psi) \\ ^{31}P: & \qquad\qquad\qquad\qquad \text{decouple} \end{array} \qquad (A)$$

$$\begin{array}{ll} ^1H: & \text{presaturate–} \quad 90_\phi\text{–}t_1/2 \quad \text{–}180_{x,sel}\text{–}180_x\text{–} \quad t_1/2\text{–}90_x \quad \text{–}90_x\text{–acq } (\psi) \\ ^{31}P: & \end{array} \qquad (B)$$

Scheme A is a variation on the heteronuclear proton-flip experiment;[3] scheme B is a selective version of the ^1H-detected heteronuclear correlation scheme.[4] The phase cycling for A is $\phi = x, y, -x, -y$ and $\psi = +, -, +, -$. For scheme B, $\phi = x, y, -x, -y$ and $\psi = +, +, -, -$, with data acquired in odd- and even-numbered scans being stored separately and processed to yield 2D absorption mode spectra.[5] For the selective pulses, we use

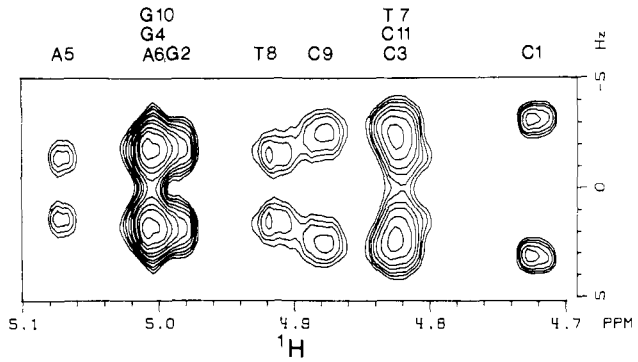

Figure 1. ^1H-detected 500-MHz 2D J spectrum of d-(CGCGAATTCGCG)$_2$ recorded with scheme A. The C3′H chemical shifts appear along the horizontal axis, with the corresponding $^3J_{HP}$ couplings along the vertical axis. An exponential line narrowing of 1.5 Hz has been used in the F_1 dimension.

low-power rectangular shapes although better results may be obtainable with shaped pulses. Since the C3′H region to be inverted is often quite close to some of the C4′H resonances, optimal setting of the selective pulse duration and rf power is important. If the C3′H region to be inverted covers a spectral width of N Hz, we recommend using an rf field strength of about N Hz with the carrier positioned in the center of the C3′H region. For scheme A, the J_{PH} coupling appears in the F_1 dimension and the resolution in this direction is determined by the ^1H T_2 value. For scheme B, all but the $J_{P\text{–}C3'H}$ couplings have been removed in the F_1 (^{31}P) dimension of the 2D correlation map. ^{31}P T_2 values are the limiting factor for resolution in this dimension.

The utility of these two methods is illustrated by applying them to a sample of the dodecamer d(CGCGAATTCGCG)$_2$ in D_2O, p^{2}H 7, 100 mM NaCl, 36 °C. Figure 1 presents a 2D J spectrum recorded with scheme A at 500 MHz. The indicated assignments of the C3′H resonances were obtained from a NOESY spectrum and are in agreement with results presented by Hare et al.,[6] except for very small changes in chemical shifts due to slightly different conditions. Only four of the C3′H resonances are resolved sufficiently to yield reliable coupling constants. Approximate values can be obtained for the partially resolved resonances of the G2 and T7 nucleotides. The nearly complete overlap of the C3′H resonances of A6/G4/G10 and C11/C3 prevents measurement of the corresponding couplings. This overlap of the ^1H resonances is removed in the correlation spectrum (Figure 2) recorded with scheme B. Because ^{31}P relaxation is dominated by chemical shift anisotropy, a considerable lengthening (by a factor of 2) of the ^{31}P T_2 was obtained by recording this spectrum at 270 MHz instead of 500 MHz. The expected loss in F_1 resolution due to the smaller chemical shift dispersion at this lower field is largely offset by the longer ^{31}P T_2 and more importantly by the decrease in the F_1 multiplet width caused by the partial decoupling provided

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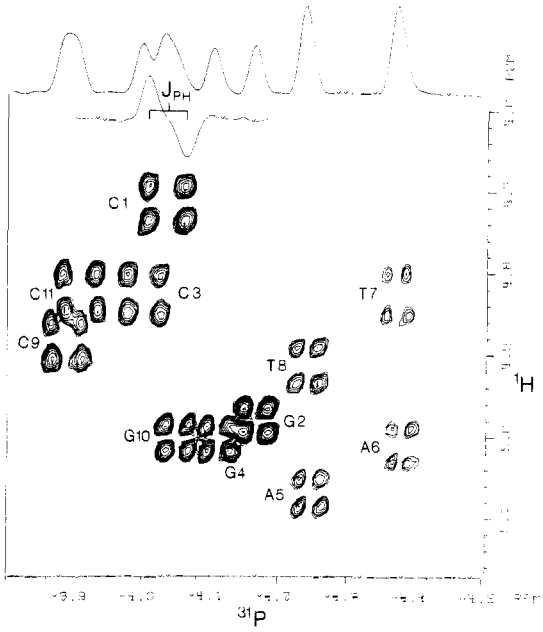

Figure 2. Partially decoupled 270-MHz ^{1}H–^{31}P correlation spectrum of the C3′H region of d(CGCGAATTCGCG)$_2$ recorded with scheme B. Both negative and positive peaks are shown. Along the top, a regular resolution-enhanced ^{31}P spectrum is shown; the inset shows a cross section through the C3′H cross peak of C1, displaying the antiphase nature of the doublet components. ^{31}P interactions to the C4′ and C5′ protons are "decoupled" in the F_1 dimension. An exponential line narrowing of 1.5 Hz has been used in the F_1 dimension. Assignment of ^{31}P resonances for which the C3′H resonances are unresolved was obtained from correlation with C4′H (supplementary material).

Table I. C3′ H–P Coupling Constants (Hz) in d(CGCGAATTCGCG)$_2$ and Dihedral ϵ Angles (Deg)

C3′H nucleotide	scheme A	scheme B	selective ^{1}H flip	ϵ_{NMR}[g]	$\epsilon_{X\text{-ray}}$[h]
C1	6.4	6.3	6.4	158	156
G2	3.7[a]	3.8	3.4	172	179
C3	*b*	5.5	5.8	161	183
G4	*b*	4.0	4.0	170	189
A5	2.8	3.3[c]	2.6[d]	178	180
A6	*b*	2.8[c]	2.3[e]	181	183
T7	2.7[a]	3.0[c]	2.3[e]	181	181
T8	3.0	3.4[c]	2.6[d]	177	187
C9	5.0	5.0	5.1[f]	163	188
G10	*b*	3.9	4.2	170	90
C11	5.2	5.2	5.1[f]	164	173

[a] C3′H resonance only partly resolved. [b] Coupling could not be determined because of overlap of the C3′ protons. [c] Overestimate of the actual size of the coupling because of partial cancellation of the antiphase doublet components. [d-f] Pairs of overlapping ^{31}P resonances. [g] Based on eq 1, using the average coupling, excluding values labeled with *a* and *c* superscripts. [h] From ref 9, averaged over the two nonequivalent strands.

by scheme B. This partial decoupling also gives a significant increase in sensitivity at the expense of the correlations to the C4′ and C5′ protons that are absent in the spectrum. As shown in the inset in Figure 2, the J_{PH} doublet splitting is antiphase. Partial cancellation of such antiphase resonances will occur if the line width is of the same order as the coupling constant. This is the case for the correlations to the C3′ protons of A6 and T7. Therefore, the J_{PH} values measured for these nucleotides represent upper limits for the actual couplings. Considering that the attenuation caused by partial cancellation within the cross peak is nearly identical for A6 and T7, the couplings must be of similar magnitude. These couplings can be measured more accurately with a selective proton-flip experiment,[3] the ^{31}P-detected version of scheme A. Couplings measured with the three different techniques are listed in Table I. In this table, the corresponding ϵ angles (obtained by adding 120° to the ϕ angles calculated from eq 1) are compared with X-ray crystallographic data, showing substantial differences.[7] A detailed structural analysis of this dodecamer, based on coupling constants and NOE buildup rates, will be presented elsewhere.[8]

The idea of measuring unresolvable couplings by suppressing other splittings, using semiselective pulses, is also applicable to the measurement of homonuclear couplings and can provide information hitherto inaccessible.

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Registry No. d(CGCGAATTCGCG)$_2$, 77889-82-8.

Supplementary Material Available: Regular ^{1}H-detected 500-MHz ^{1}H–^{31}P correlation spectrum, showing the correlations to C3′, C4′, and C5′ resonances (Figure A), ^{31}P-detected selective proton-flip experiment recorded at 270-MHz ^{1}H frequency (Figure B), and cross sections through Figure B (Figure C) (3 pages). Ordering information is given on any current masthead page.

(7) For every J_{PH} coupling measured, four possible ϕ values are obtained from eq 1. Three of these four values are discarded because they correspond to torsion angles energetically very unfavorable for B DNA.

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ESR Characterization of Ring-Closed Oxirane Radical Cations via a Novel Alternating Line Width Effect†

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There is considerable experimental evidence[1-12] supported by numerous theoretical studies[13-19] to show that the radical cation

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