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## Nuclear Overhauser Effect as a Tool for the Complete Assignment of Nonexchangeable Proton Resonances in Short Deoxyribonucleic Acid Helices<sup>†</sup>

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**ABSTRACT:** A new strategy for the assignment of nonexchangeable proton resonances in oligonucleotide duplexes is presented and used to interpret the spectra of the oligonucleotide helix d(CpGpCpGpCpG)-d(CpGpCpGpCpG) in low salt (B form). This procedure is based on the use of sequential homodecoupling (1D) or COSY (2D) for the interconnection of the sugar resonances pertaining to the same residue and on the measurement of nuclear Overhauser effect (NOE) (1D or 2D) between critically located protons in order

to establish the connectivity between the base protons and the sugar protons, as well as between consecutive base-sugar residues. The assignment performed by this method was found in perfect agreement with the one made previously by the incremental procedure [Cheng, D. M., Kan, L.-S., Frechet, D., Ts'o, P. O. P., Uesugi, S., Shida, T., & Ikehara, M. (1983) *Biopolymers* (in press)]. Also, this method is demonstrated to be applicable to DNA short helices containing A-T base pairs.

**N**uclear magnetic resonance (NMR) is one of the most powerful methods currently available for studying the conformation of nucleic acids in solution. Short oligonucleotide duplexes have been extensively used as models in such studies

[Cheng et al., 1983; Patel et al., 1982a,b; Kan et al., 1982; Kearns et al., 1981; Tran-Dinh et al., 1982a,b; Cross & Crothers, 1971; Pardi et al., 1981; Olsthoorn et al. (1980) and references cited therein]. The conformation of these short helices in solution is sufficiently close to that of native DNA to allow their recognition by enzymes (Miller et al., 1982), yet their small size makes them suitable for obtaining high-resolution NMR spectra.

In early studies, base protons, imino protons, and sugar protons were analyzed on a group basis (Cross & Crothers,

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1971; Patel et al., 1982a,b). Only recently could all the sugar and base proton resonances of a few oligonucleotide duplexes be assigned individually (Tran-Dinh et al., 1982a; Cheng et al., 1983). The assignment methods currently available rely in part on the "incremental procedure" (Borer et al., 1975; Cheng et al., 1983) and therefore can be applied only if a series of sequence-related shorter oligonucleotides is available (Borer et al., 1975; Cheng et al., 1983) or if there is no repetition in the sequence (Tran-Dinh et al., 1982a,b). The advent of a more straightforward method for the assignment of nonexchangeable proton resonances in oligonucleotide duplexes could therefore speed up the progress in our understanding of the details of the conformation and dynamics of DNA in solution by NMR spectroscopy.

Wuthrich and his collaborators have introduced a general scheme for obtaining individual resonance assignments in protein  $^1\text{H}$  NMR spectra using two-dimensional NMR (Wuthrich et al., 1982; Wider et al., 1982; Wuthrich, 1983). Although 2D spectra of nucleic acids have been obtained (Feigon et al., 1982a,b; Kaptein et al., 1982; Arndt et al., 1982), this technique has not yet been used for the systematic assignment of nonexchangeable proton resonances in nucleic acids. In this paper, we propose a general strategy to perform such assignments. This method is based on the use of sequential homodecoupling (1D) or COSY (2D) (Aue et al., 1976) for the interconnection of the sugar resonances (Kumar et al., 1980a,b, 1981; Bosch et al., 1981) pertaining to the same sugar residue and on the measurement of NOE (1D or 2D) between critically located protons in order to establish the connectivity between the base protons and sugar protons of the same nucleotidyl subunit and of the adjacent subunit. The reliability of this assignment procedure was tested by using the self-complementary helix  $d(\text{CpGpCpGpCpG}) \cdot d(\text{CpGpCpGpCpG})$ . The assignment of the nonexchangeable base and sugar protons obtained by this new procedure was found to be in perfect agreement with the assignment made previously by the incremental procedure (Cheng et al., 1983). We have also demonstrated that this strategy is applicable to DNA short helices containing A-T base pairs as well.

#### Experimental Procedures

**Materials.**  $d(\text{CpGpCpGpCpG}) \cdot d(\text{CpGpCpGpCpG})$  was synthesized and characterized previously (Uesugi et al., 1981). 150 OD units of this hexamer was lyophilized 3 times with 99.8%  $\text{D}_2\text{O}$  (Bio-Rad, Inc.) and then dissolved in 0.4 mL of  $\text{D}_2\text{O}$  containing 0.02 M phosphate buffer at pH 7.0 (pD 7.4). The final strand concentration was  $7.6 \times 10^{-3}$  M. In these conditions, the oligomer conformation belongs to the B family, as shown by circular dichroism (CD) spectroscopy, and its UV melting temperature is 70 °C (Cheng et al., 1983). The conformation of this DNA fragment has been studied previously by X-ray crystallography (Wang et al., 1979), UV spectroscopy and circular dichroism (Quadrifoglio et al., 1981; Uesugi et al., 1981), and NMR spectroscopy (Uesugi et al., 1981; Cheng et al., 1983).

**2D NOESY Spectra.** 2D NOESY experiments (Jeener et al., 1979) were performed at 25 °C on a Bruker WM-300 spectrometer located at The Johns Hopkins University (Baltimore, MD). The mixing time  $\tau_m$  was 700 ms (same order of magnitude as the average  $T_1$ ). A 1% random variation of the mixing time was used in order to eliminate  $J$  cross peaks. The spectral width was 2500 Hz. The data set consisted of 512 points in both dimensions. Twenty-four cycles of measurements were performed through all  $T_1$  values, and 16 free-induction decays (FID) per cycle were accumulated for each  $T_1$  value. This cycling procedure averages the time

instabilities and reduces the noise in the  $F_1$  direction. The relaxation delay between each pulse sequence was 3.6 s. The solvent resonance intensity was reduced by selective irradiation at the HDO frequency with a decoupler power of 15 L all the time except during the detection period. Quadrature detection was used in both dimensions. The magnitude spectrum was obtained after digital filtering in both dimensions with a cosine window.

**Sequential Homodecoupling.**  $\text{H}_1$ ,  $\text{H}_2$ ,  $\text{H}_{2'}$ ,  $\text{H}_3$ ,  $\text{H}_4$ ,  $\text{H}_5$ , and  $\text{H}_{5'}$  resonances of each individual sugar were interconnected and identified previously by sequential homodecoupling at 25 °C (Cheng et al., 1983). These experiments were performed on a Bruker WH-500 spectrophotometer located at Yale University, New Haven, CT.

**Measurement of Internuclear Distances.** Distances between neighboring protons in a B-type helix were obtained by means of our Kendrew wire model on the basis of the coordinates determined by Arnott (Arnott et al., 1972), as well as Dickerson's coordinates on CGCGAATTCBrGCG B-DNA helix (R. E. Dickerson, private communication).

#### Results

**General Strategy.** The assignment of nonexchangeable proton resonances in oligonucleotide duplexes was carried out in three steps (the first two of which are already standard procedures).

In the first step, a well-resolved 1D spectrum is obtained, and if necessary, additional experiments are performed to identify and resolve all the base proton resonances, for example, decoupling between  $\text{H}_5$  and  $\text{H}_6$  of C, selective  $T_1$  measurement, or  $\text{D}_2\text{O}$  exchange experiments in order to separate  $\text{H}_2$  and  $\text{H}_8$  (Ts'o et al., 1973; Tran-Dinh et al., 1982a,b; Cheng et al., 1983).

In the second step, the sugar proton resonances within the same nucleotidyl unit are interconnected by sequential homodecoupling (Cheng & Sarma, 1977; Cheng et al., 1983; Tran-Dinh et al., 1982a,b) or by 2D COSY (Aue et al., 1976) or SECSY (Nagayama et al., 1980). The  $\text{H}_3$ ,  $\text{H}_5$ , and  $\text{H}_{5'}$  resonances of the first and last residue can be identified because of the unique coupling pattern of these terminal protons in the absence of  $^{31}\text{P}$  splitting (see Figure 1).

In the third step, the interconnectivity between base protons and sugar protons of the same nucleotidyl subunit and of the adjacent subunit is established by nuclear Overhauser effect (NOE) measurement between critically located protons. The NOE is a dipole-dipole interaction in which magnetization is transferred through space from one proton to the other. The efficiency of the cross saturation is proportional to the inverse of the sixth power of the distance and becomes undetectable for protons that are further than 4 Å apart (Noggle & Schirmer, 1971; Bothner-By, 1979).

At this point, it is necessary to have some idea of the general conformation of the helix to be studied. A CD spectrum can indicate whether the helix belongs to the A, B, or Z family. Then, computer graphics (Kan et al., 1979; Arnott et al., 1972) or molecular models can be used to determine the most favorable interproton distances ( $<4$  Å). In the case of a B-type helix, the molecular model (Arnott et al., 1972; R. E. Dickerson, private communication) and Figure 1 show that the  $\text{H}_8$  of purines or the  $\text{H}_6$  of pyrimidines is close to the  $\text{H}_{2'}$  of their own sugar residue (approximately 2.3 and 2.0 Å, respectively). In contrast, the distance between the  $\text{H}_8$  or  $\text{H}_6$  and the  $\text{H}_2$  of the 3'-neighboring residue (Figure 1) will be larger than 4.0 Å. Moreover, the  $\text{H}_{2'}$  of the residue on the 3'-side of the phosphodiester linkage is close to the  $\text{H}_8$  of the purine (or the  $\text{H}_6$  of the pyrimidine) on the 5'-side of the phosphodiester

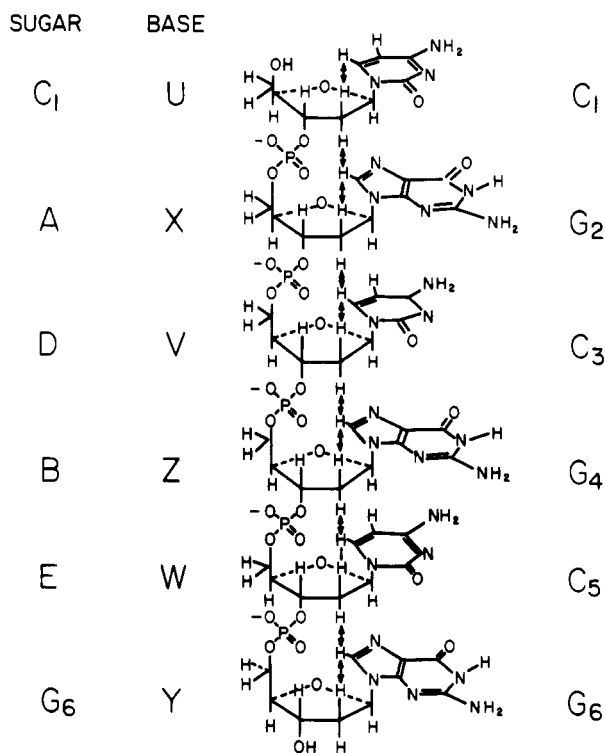


FIGURE 1: Schematic representation of a single strand of the d-(CpGpCpGpCpG)-d-(CpGpCpGpCpG) molecule. The arrows connect the protons that are located at a distance of less than 3 Å from each other.

linkage (approximately 2.8 Å). On the other hand, the distance between H<sub>8</sub> or H<sub>6</sub> and the H<sub>2'</sub> of its own sugar residue is approximately 3.0–3.5 Å.

Therefore, in order to complete our assignment, it is only necessary to measure the NOE between the base protons (H<sub>8</sub> of purine or H<sub>6</sub> of pyrimidine) and the H<sub>2'</sub> and H<sub>2''</sub> protons

of the deoxyribose. NOE can be measured either by 1D (Noggle & Shirmer, 1971) or by 2D techniques (Kumar et al., 1980a,b, 1981; Bosch et al., 1981). If the resonances are well resolved, then the 1D NOE procedure can be applied in a straightforward sequential manner starting from the 3'-end (H<sub>3'</sub> resonance) or the 5'-end (H<sub>5'</sub> and H<sub>5''</sub> resonances) of the strand, proceeding through the strand in the assignment of interconnectivity. The 2D method will be preferred if the resonances of interest are closely spaced or overlapping. Indeed, in 2D NOE methods a sequence of three nonselective 90° pulses is used in order to measure exchange of magnetization between nearby protons. Therefore, problems linked with nonselective irradiation in crowded spectral regions in 1D technique are eliminated. However, quantitative measurement of peak intensity is difficult in 2D experiments (Kumar et al., 1981). In this case, the assignment has to be obtained from the data matrix. We will now apply this general strategy to the assignment of the nonexchangeable protons of the short helix d-(CpGpCpGpCpG)-d-(CpGpCpGpCpG). The conformation of this hexamer was shown previously to belong to the B family (Cheng et al., 1983).

**(Step 1) Identification of Base Proton Resonances.** Figure 2 shows the 500-MHz spectrum of the hexamer of d-(CpGpCpGpCpG)-d-(CpGpCpGpCpG) at 25 °C. Since this sequence contains only two different types of bases, the identification of the base resonances is relatively straightforward. The three low-field singlets belong to H<sub>8</sub> of guanines, while the doublets belong to H<sub>6</sub> of cytosines. These resonances were interconnected to their respective H<sub>5</sub> by homodecoupling (Cheng et al., 1983). The H<sub>8</sub> of G bases were labeled X–Z, starting from the lowest field protons. Similarly, the H<sub>6</sub> of C bases were labeled U–W.

**(Step 2) Interconnection of Proton Resonances of the Same Residue.** Figure 2 shows the 500-MHz spectrum of the hexamer of d-(CpGpCpGpCpG)-d-(CpGpCpGpCpG) at 25 °C. Because of their unique coupling pattern (no <sup>31</sup>P splitting),

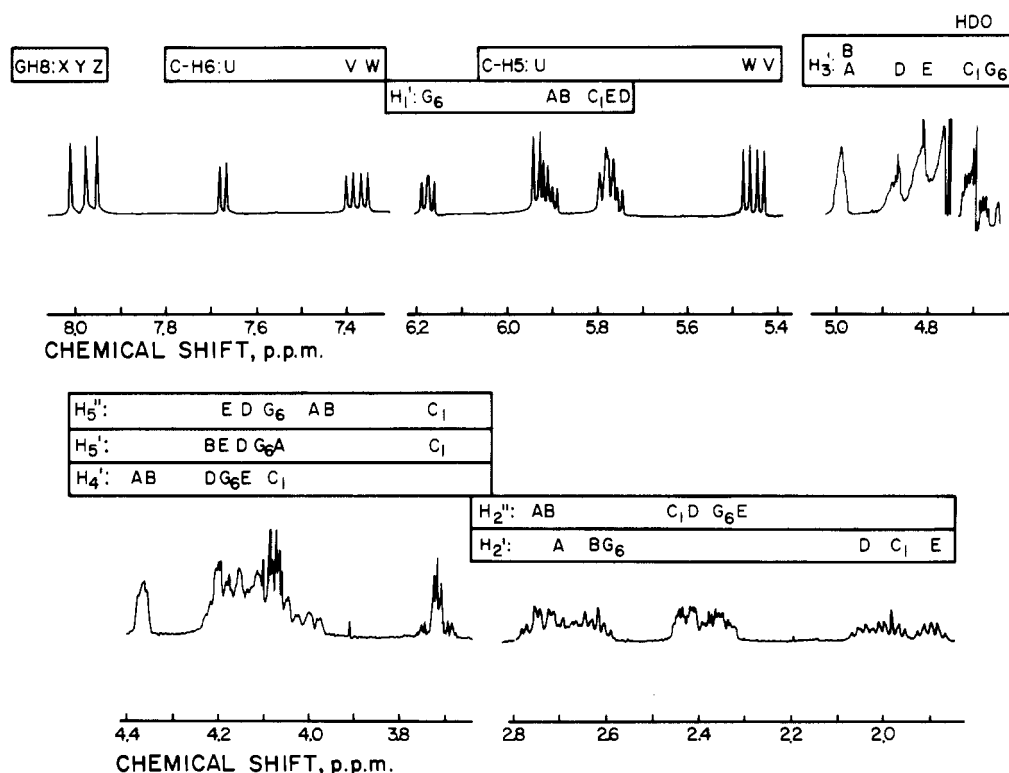


FIGURE 2: 500-MHz <sup>1</sup>H NMR spectrum of d-(CpGpCpGpCpG)-d-(CpGpCpGpCpG) at 25 °C (600 scans), 350 OD/mL. 0.02 M Na<sub>2</sub>HPO<sub>4</sub>, pD 7.4, in D<sub>2</sub>O. See Figure 1 for designations.

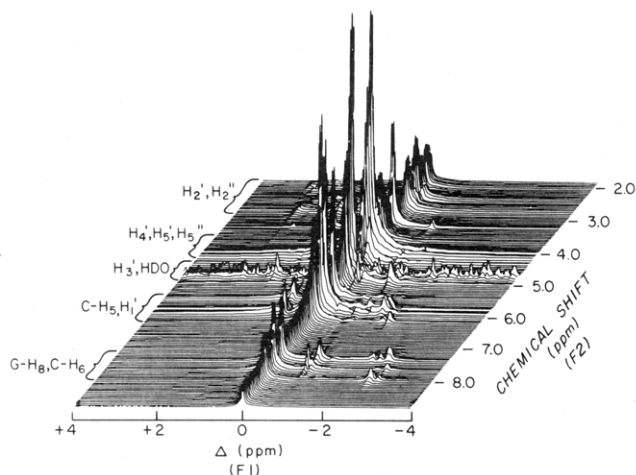


FIGURE 3: 300-MHz  $^1\text{H}$  2D NOESY stacked plot of d-(CpGpCpGpCpG)-d(CpGpCpGpCpG) (same conditions as in Figure 2). The intense peaks along the middle axis (0 ppm,  $F_1$ ) correspond to the normal one-dimensional  $^1\text{H}$  spectrum of d-(CpGpCpGpCpG)-d(CpGpCpGpCpG). Pairs of NOE cross peaks are arranged symmetrically with respect to the middle axis on a line that makes a  $45^\circ$  angle with this axis.

the resonances pertaining to the  $\text{H}_3'$  of the terminal  $\text{G}_6$  residue and to the  $\text{H}_5'$  and  $\text{H}_5''$  of the  $\text{C}_1$  residue can be easily identified. By sequential homodecoupling, the other sugar proton resonances of these terminal residues can also be assigned (Cheng et al., 1983). By use of the same method, the  $\text{H}_1'$ ,  $\text{H}_2'$ ,  $\text{H}_2''$ ,  $\text{H}_3'$ ,  $\text{H}_4'$ ,  $\text{H}_5'$ , and  $\text{H}_5''$  resonances belonging to each interior sugar residue were interconnected (Cheng et al., 1983). These interior sugar residues were labeled A, B, D, and E for the discussion at this stage. The  $\text{H}_2'$  and  $\text{H}_2''$  resonances can be distinguished from one another by their coupling pattern (Tran-Dinh et al., 1982b; Cheng et al., 1983).

**(Step 3) Nature and Sequential Position of Each Residue.** Figure 3 shows the stacked plot of the 300-MHz 2D NOESY spectrum of d-(CpGpCpGpCpG)-d(CpGpCpGpCpG) at  $25^\circ\text{C}$ . The intense peaks along the middle axis (0 ppm,  $F_1$ ) correspond to the normal one-dimensional  $^1\text{H}$  NMR spectrum of d-(CpGpCpGpCpG)-d(CpGpCpGpCpG) and arise from protons that did not cross relax from other protons during the mixing time. As expected, this spectrum shows less resolution than the one shown in Figure 2. For example, the three low-field  $\text{H}_8$  of G are clustered together in a single envelope, but they can be located by their chemical shifts. Pairs of NOE cross peaks are arranged symmetrically with respect to the middle axis, on a line that makes a  $45^\circ$  angle with the middle axis. Therefore, the position of the cross peak  $\delta_{\text{cp}}$  can be determined by simple trigonometric relations:  $\delta_{\text{cp}} = \delta_0 + 2\Delta$  where  $\delta_0$  is the position of the peak on the middle axis ( $F_2$  dimension) and  $\Delta$  is the spectral position of the cross peak in the  $F_1$  dimension. Because of the large number of overlapping resonances, it is difficult to locate precisely the position of the NOE cross peaks by examination of the stacked plot in Figure 3. This could be done more easily by examination of the cross sections corresponding to the resonances of interest.

From the scheme shown in Figure 1, we can set up a strategy in order to complete the assignment of the nonexchangeable proton resonances of the hexamer d-(CpGpCpGpCpG)-d(CpGpCpGpCpG). The first step will be to establish the connectivity between each base  $\text{H}_8$  or  $\text{H}_6$  proton and the  $\text{H}_2'$  of the sugar from the same residue. The second step will be to establish interresidue connectivity by examining the NOE cross peaks between  $\text{H}_2''$  of the sugar and  $\text{H}_8$  or  $\text{H}_6$  of the base from the adjacent residue on the 5'-side of the phosphodiester linkage. We will therefore only examine cross

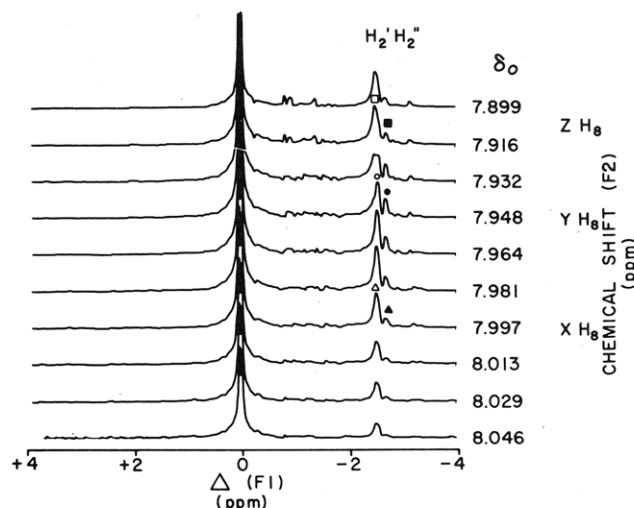


FIGURE 4:  $F_1$  cross sections corresponding to the three  $\text{G H}_8$  resonances X-Z. The chemical shift of the cross peaks  $\delta_{\text{cp}}$  can be obtained by the simple trigonometric relation  $\delta_{\text{cp}} = \delta_0 + 2\Delta$  (see text for definition): ( $\Delta$ ) 2.73 ppm; ( $\blacktriangle$ ) 2.38 ppm; ( $\circ$ ) 2.64 ppm; ( $\bullet$ ) 2.38 ppm; ( $\square$ ) 2.71 ppm; ( $\blacksquare$ ) 2.38 ppm.

peaks between base protons and sugar protons in the  $\text{H}_2'$  and  $\text{H}_2''$  region.

In Figure 4, the cross sections corresponding to the three  $\text{H}_8$  proton resonances of G are shown. From the inter proton distances described in the preceding section, we would expect that each  $\text{H}_8$  of G will have a major NOE to the  $\text{H}_2'$  of its own sugar residue ( $\approx 2.0 \text{ \AA}$ ) and smaller NOE to the  $\text{H}_2''$  of the adjacent residue on the 3'-side of the phosphodiester linkage ( $\approx 2.8 \text{ \AA}$ ) and to the  $\text{H}_2''$  of the sugar from its own sugar residue ( $\approx 3\text{--}3.5 \text{ \AA}$ ). As shown in Figure 4, resonance X (7.997 ppm) presents a large cross peak at 2.74 ppm and a smaller one around 2.38 ppm, resonance Y (7.948 ppm) has its major cross peak at 2.64 ppm and a smaller one at 2.38 ppm, and resonance Z (7.916 ppm) has a large cross peak at 2.71 ppm and a minor one at 2.38 ppm. The results of the homodecoupling experiments indicate that the  $\text{H}_2'$  resonances of sugars A, B, and  $\text{G}_6$ , as well as the  $\text{H}_2''$  resonances of sugars A and B, are located in the 2.6–2.8 ppm frequency range. In this region, the lowest field  $\text{H}_2'$  resonance belongs to sugar A, whereas the  $\text{H}_2'$  resonance at intermediate field belongs to sugar B and the  $\text{H}_2'$  resonance at the highest field belongs to sugar  $\text{G}_6$ . Therefore, base X is connected to  $\text{H}_2'$  in sugar A, base Y is connected to  $\text{H}_2'$  in sugar  $\text{G}_6$ , and base Z is connected to  $\text{H}_2'$  in sugar B. The homodecoupling experiments also indicate that the  $\text{H}_2''$  resonances of the same sugars, whereas the  $\text{H}_2''$  resonances of sugars  $\text{G}_6$ ,  $\text{C}_1$ , D, and E are all in the 2.33–2.45 ppm region. This observation accounts for the fact that each  $\text{G H}_8$  resonance has only two distinguishable cross peaks. This makes it difficult to determine which sugar residue is adjacent to which base on the basis of these spectra alone. However, these difficulties can be overcome by examination of other cross sections of the 2D experiment.

The cross sections corresponding to the three  $\text{H}_6$  of C resonances, U–W, are shown in Figure 5. Resonance U (Figure 5a) is associated with a major cross peak at 1.99 ppm and a minor one at 2.44 ppm (1.64 ppm peak due to ammonium salt impurity). Resonance V (Figure 5b) is associated with a major peak at 2.07 ppm and two smaller ones at 2.81 and 2.48 ppm. Resonance W (Figure 5b) has a major cross peak at 1.87 ppm and two minor ones at 2.73 and 2.37 ppm. From the homodecoupling experiments, we know that in the region between 1.9 and 2.1 ppm, the  $\text{H}_2'$  resonances of sugars D,  $\text{C}_1$ , and E

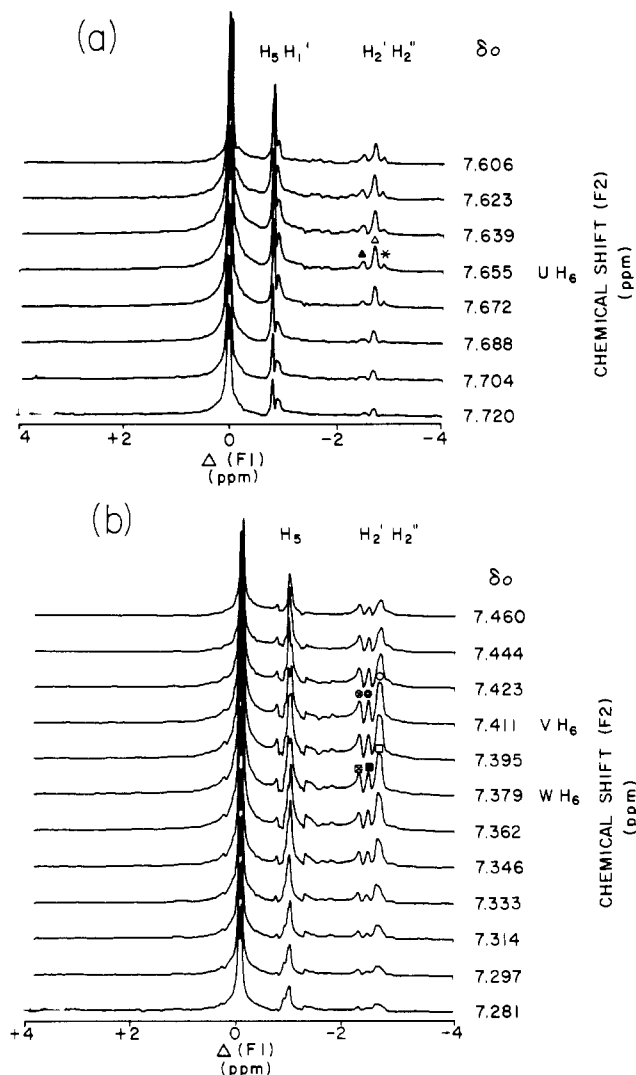


FIGURE 5:  $F_1$  cross sections corresponding to the three  $CH_6$  resonances: U (a) and V and W (b).  $\delta_{op}$  obtained as described in Figure 4. (a) ( $\blacktriangle$ ) 2.44 ppm; ( $\triangle$ ) 1.99 ppm; ( $*$ ) 1.635 ppm. (b) ( $\otimes$ ) 2.81 ppm; ( $\bullet$ ) 2.43 ppm; ( $\circ$ ) 2.07 ppm; (crossed box) 2.73 ppm; ( $\blacksquare$ ) 2.37 ppm; ( $\square$ ) 1.87 ppm.

appear at low-, intermediate-, and high-field position, respectively. Thus, base U is connected to  $H_{2'}$  in sugar  $C_1$ , base V is connected to  $H_{2'}$  in sugar D, and base W is connected to  $H_{2'}$  in sugar E. As far as the minor signals are concerned, they should belong to the  $H_{2'}$  of the same residue and the  $H_{2'}$  of the adjacent residue in the 5'-direction, and it therefore has only one minor cross peak at 2.44 ppm, which arises from the  $H_{2'}$  of its own sugar, as confirmed by homodecoupling experiments (Figure 2). Base V (sugar D) has two minor cross peaks: one at 2.81 and one at 2.43. The 2.43 ppm peak belongs to the  $H_{2'}$  of its own sugar residue (D) as seen by homodecoupling experiments. Therefore, the peak at 2.81 ppm is associated with the residue adjacent to it in the 5'-direction. Base W (sugar E) has minor cross peaks at 2.73 and 2.37 ppm. By similar arguments, we can show that the  $H_{2'}$  resonance at 2.37 ppm belongs to the same sugar residue (sugar E) and the one at 2.73 ppm belongs to the adjacent residue in the 5'-direction. The homodecoupling experiments reveal that in the 2.6–2.85 ppm frequency range, the  $H_{2'}$  proton of sugar A (associated with base X) has the lowest field resonance whereas sugar B (associated with base Z) resonates at slightly higher field. Therefore, the  $H_6$  proton of base V, which has a cross peak at 2.81 ppm, must be close ( $<3$  Å) to the  $H_{2'}$

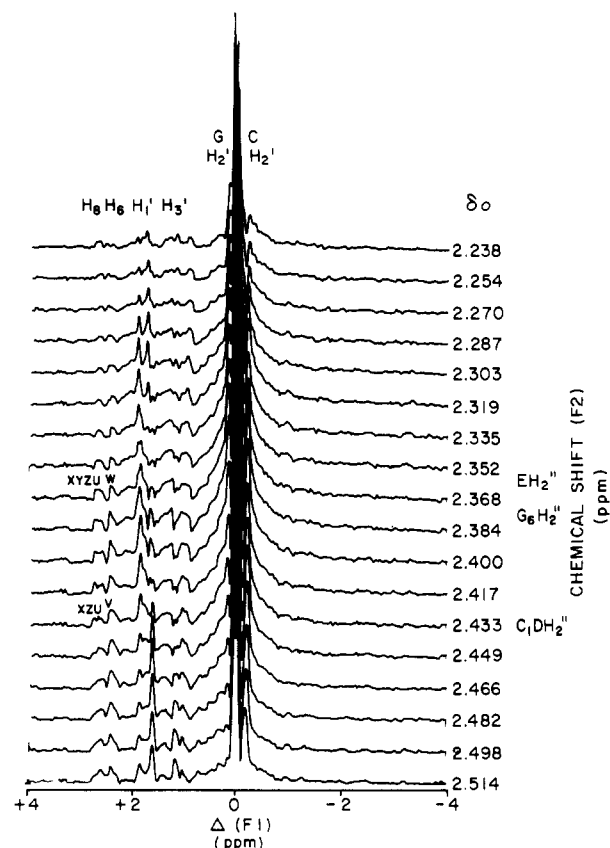


FIGURE 6:  $F_1$  cross sections corresponding to the  $H_{2'}$  protons of sugars E,  $G_6$ ,  $C_1$ , and D. The letters on top of the peaks refer to the resonances of the  $H_8$  protons of guanines (X, Y, Z) and of the  $H_6$  protons of cytosine (U, V, W).

proton of sugar A (base X), whereas the  $H_6$  proton of base W, which has a cross peak at 2.73 ppm, must be close ( $<3$  Å) to the  $H_{2'}$  of sugar B (base Z). Figure 1 and the information on inter hydrogen distance show that the  $H_6$  protons of cytosine should be close to the  $H_{2'}$  proton ( $\approx 2.8$  Å) of the residue adjacent in the 5'-direction, we can conclude that the sequence is either model I or II:

sugar:	$C_1$	A	D	B	E	$G_6$	$C_1$	B	E	A	D	$G_6$
base:	U	X	V	Z	W	Y	U	Z	W	X	V	Y
	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$
	$C_1$	$G_2$	$C_3$	$G_4$	$C_5$	$G_6$	$C_1$	$G_2$	$C_3$	$G_4$	$C_5$	$G_6$
	model I						model II					

In order to complete the assignment, it is necessary to look at the cross sections in the  $H_{2'}$  region. Figure 6 shows the cross sections corresponding to the peaks between 2.3 and 2.5 ppm. Sugars  $C_1$  (associated to base U) and D (associated to base V) have their  $H_{2'}$  resonances in the 2.43–2.44 ppm region whereas sugars E (associated to base W) and  $G_6$  (associated to base Y) have their  $H_{2'}$  resonances in the 2.33–2.38 region. It can be shown that  $H_{2'}$  of sugar  $C_1$  and  $H_{2'}$  of sugar D have cross peaks to base resonances X, Z, U, and V, whereas the  $H_{2'}$  resonances of sugars  $G_6$  and E present additional cross peaks to bases Y and W resonances (despite some overlap with the previous signals). This can be seen more clearly from Figure 7, which shows the enlargements of the base proton regions of the cross sections at 2.433 ppm (a) and at 2.368 ppm (b). The cross section of 2.433 ppm corresponds to the  $H_{2'}$  protons of sugars  $C_1$  and D. They show cross peaks to bases X, Z, U, and V. The data presented previously have shown that sugar  $C_1$  is connected with base U and sugar D is connected with base V. Therefore, cross peaks X and Z must

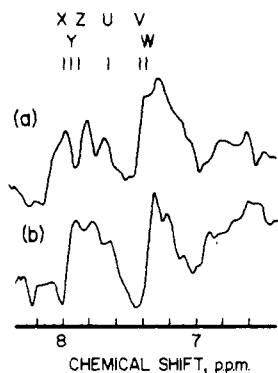


FIGURE 7: Enlargement of the base region of the cross sections at 2.433 (a) and at 2.368 ppm (b). The scale has been converted to absolute chemical shift. The lines at the top of the figure indicate the position of the H<sub>8</sub> resonances of bases X, Y, and Z and of the H<sub>6</sub> resonances of bases U, V, and W.

come from the base of the residues adjacent to sugars C<sub>1</sub> and D in the 3'-direction. Therefore, the base following sugar C<sub>1</sub> (associated with base U) must be either X or Z, and the base following sugar D must also be either X or Z. While the first observation is compatible with both models I and II, the second one rules out model II. Therefore, we can conclude that the sequence is

sugar:	C <sub>1</sub>	A	D	B	E	G <sub>6</sub>
base:	U	X	V	Z	W	Y
	↑	↑	↑	↑	↑	↑
	C <sub>1</sub>	G <sub>2</sub>	C <sub>3</sub>	G <sub>4</sub>	C <sub>5</sub>	G <sub>6</sub>

## Discussion

The present investigation shows that it is possible to use 2D NOE measurements in conjunction with homodecoupling or 2D COSY spectroscopy in order to make a complete assignment of all the nonexchangeable proton resonances in an oligonucleotide duplex. The assignments are in perfect agreement with those previously made by the incremental procedure (Cheng et al., 1983). This new approach is less time consuming and demanding on materials than the previous approach based on the incremental procedure, since it does not require previous knowledge of the spectra of sequence related shorter oligonucleotides and it can be done at a single temperature. Although the experiments presented here were done with a large number of scans (24 cycles of 16 FID), in preliminary experiments four cycles gave a reasonable signal to noise ratio. Moreover, in many cases it should be possible to record only the base proton region in the F<sub>1</sub> dimension of the 2D spectrum and to fill the rest of the spectrum with zero values before the Fourier transform, thereby further shortening the data-acquisition time.

In this paper, NOE measurements were used to assign the proton resonances of an oligonucleotide helix containing only cytosine and guanine bases. This method can be applied to the assignment of proton resonances of oligonucleotide helices containing also adenine and thymine. Thus, two concerns will be taken into consideration: (i) the connection of H<sub>2</sub> of A to the sugar protons and (ii) the NOE effects between H<sub>6</sub> of T to the sugar protons in the presence of T CH<sub>3</sub> group.

Adenine has two nonexchangeable protons, H<sub>2</sub> and H<sub>8</sub>. The resonances of these two protons can be distinguished from each other by their longitudinal relaxation times T<sub>1</sub>. Indeed, the T<sub>1</sub> of H<sub>2</sub> is much greater than the T<sub>1</sub> of H<sub>8</sub> (Ts'o et al., 1973; Tran-Dinh et al., 1982a,b). H<sub>8</sub> of adenine, like H<sub>8</sub> of guanine, can be connected to the H<sub>2'</sub> of the sugar of the same residue and the H<sub>2''</sub> of the sugar of the adjacent residue in the 5'-

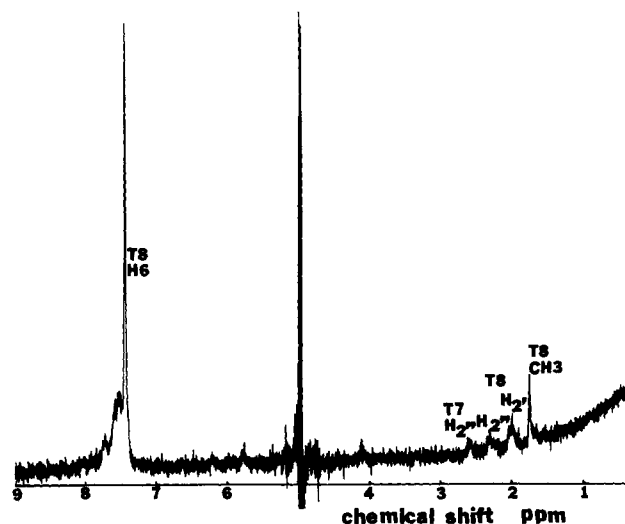


FIGURE 8: 1D NOE difference spectrum of the oligonucleotide helix shown below upon irradiation of the H<sub>6</sub> resonance of T<sub>8</sub> (temperature

1	2	3	4	5
C	C	A	A	G
G	G	T	T	C
10	9	8	7	6

5 °C; number of scans 7600; decoupling power 30 L; relaxation delay 3 s; NOE buildup delay 0.5 s) [assignment from L.-S. Kan et al. (unpublished results)]. These data demonstrate the NOE effect of H<sub>6</sub> of T to CH<sub>3</sub> of T, to the H<sub>2'</sub> and H<sub>2''</sub> of the same residue, and to the H<sub>2''</sub> of the neighboring residues.

direction by NOE measurement. On the other hand, H<sub>2</sub> of adenine is not close (<4 Å) to any nonexchangeable proton. The H<sub>2</sub> resonances of adenine can, however, be assigned in H<sub>2</sub>O through NOE with the NH-N resonances of the same A·T base pair, since NH-N proton resonances can be assigned readily by the thermal-perturbation method (Kan et al., 1982). The validity of this approach has been confirmed previously (Kan et al., 1982).

Thymine has four nonexchangeable protons: H<sub>6</sub> and the three equivalent protons of the methyl group in position 5. The methyl group protons can be assigned by NOE measurement upon irradiation of H<sub>6</sub> of T (Figure 8). The H<sub>6</sub> of T, like H<sub>6</sub> of C, can be connected to the H<sub>2'</sub> of the sugar of the same residue and H<sub>2''</sub> of the sugar of the adjacent residue in the 5'-direction by NOE measurement, provided it is not entirely relaxed by the CH<sub>3</sub> group. This approach has been confirmed by our NOE measurements between the H<sub>6</sub> of T and the H<sub>2'</sub> and H<sub>2''</sub> protons in the oligonucleotide duplex CCAAG/GGTTC (Figure 8).

Since this approach is based on a significant NOE observable, the dependence of the NOE intensity on  $\omega\tau_c$  ( $\omega$  being the Larmor frequency and  $\tau_c$  the correlation time) should be taken into account. It has been shown that as long as  $\omega\tau_c$  is smaller than 0.1, the NOE intensity is maximum (~50%). As  $\omega\tau_c$  increases, the NOE intensity decreases and then becomes negative. The change from positive to zero and to negative NOE occurs at  $\omega\tau_c = 1.118$  (Bothner-By, 1979). In this particular case, it might be necessary to work at a different field strength, to vary the temperature, or to use another solvent in order to be able to observe the NOE for assignment purposes.

In this paper, the NOE measurements were used only on a qualitative basis. We believe that it would be interesting to perform 2D measurements at different mixing times in order to determine the precise geometry of the oligomer and to elucidate the cross-relaxation pathways. This work is now in progress.

## Acknowledgments

We thank the JHU-Baltimore Biomedical NMR Center for the use of the Bruker WM-300 (established by Grant GM 27512). We are grateful to Professor M. Ikehara, who provided us with the oligomer d(CpGpCpGpCpG)-d-(CpGpCpGpCpG).

**Registry No.** d(CpGpCpGpCpG), 58927-26-7; CCAAG-CTTGG, 87136-39-8.

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