Conformational Study of Two Short Pentadeoxyribonucleotides, d-CpCpApApG and d-CpTpTpGpG, and Their Fragments by Proton Nuclear Magnetic Resonance†

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ABSTRACT: Two pentadeoxyribonucleotides, d-CpCpApApG and d-CpTpTpGpG, and their short fragments, d-CpCpA, d-CpCpApA, d-TpGpG, and d-TpTpGpG, were synthesized by the phosphodiester or phosphotriester method and have been studied thoroughly by ^{1}H NMR spectroscopy. All the nonexchangeable base proton resonances (about 8–10 per each pentamer) of these two series of oligodeoxynucleotides have been assigned by the "incremental method". The chemical shift and coupling constant values of the nonexchangeable sugar proton resonances (35 per each pentamer) have been determined by spectral simulation method, and the assignments of these 35 resonances were achieved by both the "incremental method" and the "sequential homodecoupling" method. Through the chemical shift vs. temperature profile, the assignments of the proton resonances at one temperature (usually at high temperature, i.e., 75 °C) can be extended to other temperatures. Thus, together with other NMR techniques, the strategy of (i) incremental procedure, (ii) sequential homodecoupling techniques, and (iii) chemical shifts vs. temperature measurement is now shown to be effective for total assignment of all resolvable proton resonances in oligonucleotides. The coupling constants of these known sugar proton resonances provide valuable information about the backbone conformation, particularly with respect to the influence of chain length and base composition of these oligodeoxynucleotides. The sugar conformation was shown to be predominantly in the ^2E form (over 65%). The addition of 5′-deoxyadenylic acid and 5′-deoxyguanylic acid to the 3′-OH of a nucleoside is more effective in increasing the percent ^2E of the nucleoside than the addition of 5′-deoxycytidylic acid and 5′-thymidylic acid. On the other hand, the 3′-nucleotidyl unit exerts little effect on the sugar conformation of the 5′-nucleosidyl unit. Thus, the residue at the 3′ end (with free 3′-OH group) always has a lower percent ^2E than the internal residues adjacent to a 5′-purine nucleotidyl unit. The rotation of the C_4–$C_{5'}$ bond (ψ angle) is in favor of the *gg* conformation (55–85%), the rotation of the $C_{5'}$–$O_{5'}$ bonds (ϕ angle) is highly in favor of *g′g′* (75–90%), and the rotation of the C_3–$O_{3'}$ bond (ϕ' angle) is restricted to the domain of $\phi \simeq 200°$. Also, the percent *gg* of the nucleotidyl unit of the free 3′ end are higher than those of the free 5′ end, indicating the restricted rotation of the C_4–$C_{5'}$ bond of the 3′ end. The population distributions of these three bonds, ψ, ϕ, and ϕ', are relatively insensitive to temperature (25–75 °C), indicating that the rotation of these angles is considerably restricted to their favored conformations. The achievement in the complete assignments of all nonexchangeable protons of these two complementary pentamers paves the way for assignment of all the protons of the helical duplex of d-(CCAAG + CTTGG) as well as assignment of the ^{13}C resonances and ^{31}P resonances for these two pentamers by the heterodecoupling technique.

Studies of the physical properties of short double helices of oligonucleotides by NMR[1] have been the subject of considerable recent interest (Cross & Crothers, 1971; Arter et al., 1974; Patel & Canuel, 1979; Kallenbach et al., 1976; Borer et al., 1975; Phillips & Roberts, 1980; Pardi et al., 1981). The thermal behavior of the chemical shifts for the exchangeable and nonexchangeable protons of the bases provides information useful for characterizing the oligomers in the helix or coil states. More detailed information on the geometrical and motional properties of the helical duplex awaits more accurate knowledge of the chemical shifts and coupling constants for the sugar protons and the changes which these parameters undergo during a melting transition. Such information should make possible a detailed evaluation of the sugar pucker and rotamer population for an oligomer duplex at least to the size of the pentamer. Then, it would be of interest to know whether these conformational properties are conserved as the duplex is extended in length. Due to their smaller size, short helices offer advantages over the larger helical molecules for study by NMR. Together with the greater availability of oligodeoxyribonucleotides due to recent developments in synthetic methods, short helices should become useful models for studying structure and conformation of nucleic acid in solution.

Recently, the base proton resonances in the ^{1}H NMR spectrum of d-CpCpApApGpCpTpTpGpG have been resolved at 360 and 600 MHz and have been assigned (Miller et al., 1980) at high temperature. These assignments were made by an incremental procedure according to Borer et al. (1975) using the smaller fragments d-CpCpA, d-TpGpG, d-CpCpApA, d-TpTpGpG, d-CpCpApApG, and d-CpTpTpGpG. In this paper we report the reliable assignment of *all* the base proton resonances and nearly all sugar proton resonances in the 360-, 500-, and 600-MHz NMR spectra of the single-stranded trimers, tetramers, and pentamers. The pentose protons of A^3 and A^4 in d-CpCpApApG have very similar chemical shifts. Therefore, the assignments of these resonances are less certain than the assignments of other resonances. The chemical shifts and coupling constants were obtained. The

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[1] Abbreviations: NMR, nuclear magnetic resonance; d-NpN, a deoxyribooligonucleotide *p*-chlorophenyl phosphotriester; MST, mesitylenesulfonyl tetrazolide; bz, benzoyl; ibu, isobutyryl; [$(MeO)_2$Tr], dimethoxytrityl. The symbols used to represent oligonucleotides and their protected derivatives follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (1970).

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sugar and backbone conformations were analyzed by computer simulation techniques.

Experimental Procedures

Syntheses of Oligodeoxyribonucleotides. The materials and methods used to prepare the oligodeoxyribonucleotides are similar to those described previously (Miller et al., 1980). Detailed procedures are provided in the supplementary material (see paragraph at end of paper regarding supplementary material).

NMR Measurements. All NMR samples were lyophilized 3 times with 99.8% D_2O (Bio-Rad, Inc.) and then dissolved in D_2O contained 0.01 M phosphate buffer at pH 6.5. The ^1H NMR spectra were obtained from three different spectrometers. The complete spectra of d-CpCpA, d-TpGpG, d-CpCpApA, and d-TpTpGpG as well as the base proton resonances of d-CpCpApApG and d-CpTpTpGpG were recorded on a Bruker WH-360 NMR spectrometer (magnetic field strength 8.4 T) located at the University of Pennsylvania, Philadelphia, PA. This spectrometer is equipped with FT and variable temperature accessories. The sugar proton resonances of two pentamers and all homodecoupling experiments of these resonances were performed by a home-built 500-MHz NMR spectrometer (11.8 T) located at the Francis Bitter National Magnet Laboratory, Cambridge, MA. However, the spectra of the sugar proton resonances of these two pentamers in Figure 6 were recorded by a home-built 600-MHz NMR spectrometer located at Carnegie-Mellon University, Pittsburgh, PA. This spectrometer is operated by fast correlation spectroscopy (Dadok & Sprecher, 1974) with the HDO signal as a lock. The probe temperature is fixed at 20 °C.

The spectrum simulation was performed on a JEOL 980 minicomputer with JEOL 6-spin simulation program and Nicolet 1180 minicomputer.

Results and Discussion

Synthesis. The fully protected oligodeoxyribonucleotides, d-[$(MeO)_2$Tr]bzCṗbzCṗbzA$_{OAc}$, d-[$(MeO)_2$Tr]-bzCṗbzCṗbzAṗbzAṗibuG$_{OAc}$, and d-[$(MeO)_2$Tr]-bzCṗTṗTṗibuGṗibuG$_{OAc}$, were synthesized by the triester method. The strategy in these syntheses involved the preparation of the triester intermediates (Miller et al., 1980) d-[(MeO_2)Tr]bzCṗbzCṗ, d-[$(MeO)_2$Tr]bzCṗbzCṗbzAṗbzAṗ, and d-[MeO_2)Tr]bzCṗTṗTṗ where ṗ symbolizes the *p*-chlorophenyl phosphate group. Subsequent condensation with d-bzA$_{OAc}$ and d-ibuGṗibuG$_{OAc}$, respectively, afforded the protected trimer and two pentameters. The base and phosphate protecting groups were removed from d-[$(MeO)_2$Tr]-bzCṗbzCṗbzA$_{OAc}$ by treatment with concentrated NH_4OH–pyridine (40:1) nixture (Stawinski et al., 1977) at 50 °C overnight. Under these conditions, the 3′-*O*-acetyl group was also removed. The tritylated product, d-[$(MeO)_2$Tr]CpCpA, was separated from the reaction mixture by chromatography on paper. A 46% yield (0.02 mmol) of d-CpCpA was obtained, after removal of the dimethoxytrityl group, separation by paper chromatography, and final purification by chromatography on DEAE-cellulose. For the longer oligomers, the pyridine-2-aldoximate reagent (Reese et al., 1978) was used, since it seemed to give the smallest amount of degradation products when compared to other deprotection methods. The protected oligomers, d-[$(MeO)_2$Tr]bzCṗbzCṗbzAṗbzAṗibuG$_{OAc}$ and d-[$(MeO)_2$Tr]bzCṗTṗTṗibuGṗibuG$_{OAc}$, were each treated with pyridine-2-aldoximate reagent for approximately 15 h, which effectively removed the *p*-chlorophenyl protecting group. The excess aldoximate reagent was removed from the reaction products by chromatography on DEAE-cellulose. Final treatment with NH_4OH–pyridine (40:1) at 50 °C for 2 h followed by 80% HOAc at room temperature for 15 min resulted in the removal of the base and sugar protecting groups. The crude pentanucleotide oligomers were purified by preparative high-pressure liquid chromatography (HPLC) on Pellinoex AL wax by using a linear gradient of NH_4OAc in 60% ethanol. The oligomers were freed of the NH_4OAc by precipitation from 95% ethanol in which NH_4OAc was soluble. The precipitated oligomers were collected and further chromatographed on small columns of DEAE-cellulose to give pure d-CpCpApApG (227 OD_{257}, 44% yield) and d-CpTpTpGpG (140 OD_{257}, 26% yield). d-(MeOTr)bzCpanC and d-pbzApbzA$_{OAc}$ were prepared according to published procedures (Kumar & Khorana, 1972) and were condensed in the presence of triisopropylbenzenesulfonyl chloride. After the reaction was complete as ascertained by HPLC analysis, the condensation mixture was fractionated by column chromatography on DEAE-cellulose to afford a 48% yield (0.3 mmol) of the fully protected tetramer. The base and sugar protecting groups were removed by standard methods except that the treatment with concentrated ammonium hydroxide was done at elevated temperatures in a sealed tube to ensure complete removal of the benzoyl and anisoyl groups. Final purification by paper and DEAE-Sephadex chromatography gave 0.04 mmol (33% yield) of pure d-CpCpApA.

The synthetic oligomers prepared by the diester and triester methods were homogeneous as shown by paper chromatography and HPLC (Pellionex AL wax and ODS-2). Further indications of purity were shown by NMR and by digestion with snake venom phosphodiesterase. The NMR spectra for d-CpCpApApG and d-CpTpTpGpG indicated that no *p*-chlorophenyl phosphate protecting groups remained on the oligomers deprotected by the pyridine-2-aldoximate reagent. HPLC analysis (reverse phase, ODS-2) of the enzymic digests revealed no other hydrolysis products except those expected (Table IX in supplmentary material).

Extreme care was taken in the preparation and storage of NMR samples of the oligomers. The oligomer samples were prepared in autoclaved water so as to prevent biological contamination. The oligomers were stored at −80 °C either as the lyophilized solid or as frozen aqueous solutions. The buffer solutions were passed through a 0.22-μm filter. The NMR samples were analyzed by HPLC before and after the NMR experiment to ensure the integrity of the oligonucleotides.

^1H NMR Studies of d-CpCpApA, d-TpTpGpG, d-CpCpApApG, and d-CpTpTpGpG and Their Smaller Fragments. (*a*) *Assignments of the Base Proton Resonances.* The assignment of base proton resonances of d-CpCpApApG and d-CpTpTpGpG can be readily accomplished by the "incremental procedure" (Borer et al., 1975). As illustrated in Figure 1, the assignment of the two pentamers starts from the dimeric units. In d-CpTpTpGpG, the assignment is straightforward. In d-CpCpApApG, the assignment of H_2, H_8 of adenine base and H_8 of guanine is also straightforward (Figure 1). However, the assignment of two C-H_6 resonances are reversed as compared to those in d-CpC. This is due to the ring current shielding effect from the two neighboring adenine bases in the pentamer. The complete argument and detailed assignment of each base proton resonance of these two pentamers are given as follows:

H_5 and H_6 of Cytosine. The pentamer of d-CpTpTpGpG contains only one cytosine, therefore the only doublet (J = 7.2 Hz) at the base region can be readily assigned to H_6 of C. The assignment of two H_6 resonances of d-CpCpApA can be achieved by the paramagnetic ion broadening method (Ts'o

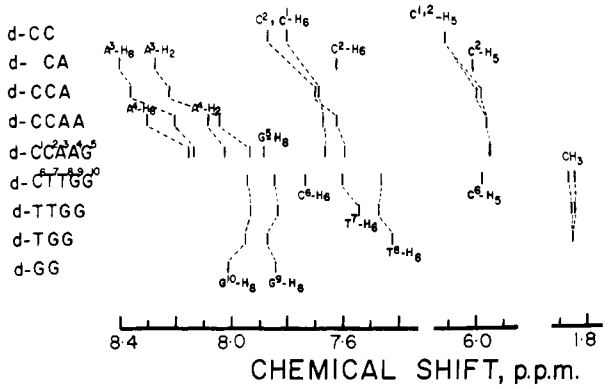

FIGURE 1: Incremental assignment scheme of the base proton resonances of deoxyoligonucleotides from dimeric to pentameric units at 75 °C.

et al., 1969). The doublet at higher field became broadened when a trace of Mn^{2+} was introduced. Therefore, this broadened doublet can be assigned to the H_6 of C^2 (Figure 1; Ts'o et al., 1969). The assignment of H_5 resonances in d-CpCpApA can be easily made by decoupling the assigned H_6 signals. The result is also shown in Figure 1.

It is interesting to note that the assignment of two H_6 resonances in d-CpCpApA is just opposite to that in d-CpC, an assignment also accomplished by the paramagnetic ion broadening method (Figure 1). This is because the C^2 base in the tetramer is shielded by the A^3 and A^4 bases. Other supporting evidence can be obtained from d-CpCpA and d-CpA. Even though the downfield doublet in d-CpCpA is still assigned to the H_6 of C^2 (same as in d-CpC), it is significantly shifted upfield as compared to d-CpC and resonates close to H_6 of C^1 [which is also shifted upfield compared to d-CpC but by a smaller amount (Figure 1)]. Furthermore, this downfield doublet (C^2-H_6) shifts to higher field position than that of C^1 at low temperature (Figure 2a). These facts strongly indicated the shielding effect of the A^3 base. Therefore, the H_6 resonance of C^2 in d-CpCpApA can receive the ring-current shielding effect from the A^4 as well the A^3 base and resonates at a higher field position (Kan et al., 1973a) (Figure 1). In d-CpA, the H_6 resonance of cytosine base (doublet) has the same chemical shift values as C^2-H_6 in d-CpCpApA. This indicates that the C-H_6 is shifted upfield by the adenine base. The higher field position of C^2 in d-CpCpA, as compared to d-CpC, may be caused by the reduction of internal base rotation in the trimer, as opposed to the dimer (Kan et al., 1973b). The assignment of all H_5 resonances are determined by decoupling H_6 doublets.

H_2 and H_8 of Adenine. The identities of H_8 and H_2 can be distinguished by their spin–lattice relaxation time (T_1). Since the H_8 proton is in close proximity to the sugar unit when the nucleosidyl unit is in the anti conformation, the T_1 of H_8 is relatively shorter than that of H_2. Thus, the two singlets in d-CpCpA can be readily assigned (Figure 1). The H_2 and H_8 resonances of d-CpCpApA again can be separated by their T_1 values. The set (H_2 and H_8) located at higher field is assigned to A^3, because it is shielded by A^4. The argument was presented in the previous section. This assignment can be extended to d-CpCpApApG at high temperature (Figure 1). Even though H_8 resonances of A^3 and A^4 are only 0.02 ppm apart, the reverse assignment can be ruled out by a careful consideration of the ring-current shielding effect. Since the ring-current shielding effect of guanine is known to be less than that of adenine (Giessner-Prettre et al., 1976), the upfield shift of A^4-H_8 from the extension of d-CpCpApA to d-CpCpApApG (0.15 ppm) should be smaller than that of A^3-H_8 from the extension of d-CpCpA to d-CpCpApA (0.165 ppm). Similar results are also obtained for A-H_2 resonances (Figure 1) in a comparison between A^3-H_2 and A^4-H_2 upon increases in chain length. If the assignment is reversed, then the ring current effect of addition of a guanine residue would be slightly larger than that of the addition of an adenine residue, a consequence not supported by experimental observation and theoretical calculation.

H_8 of Guanine. The assignment of the two H_8 resonances in d-TpTpGpG derives from that of d-GpG, which is determined by the paramagnetic ion broadening method. The result is shown in Figure 1. Two singlets with chemical shift values similar to those of d-GpG are observed in d-TpGpG and d-TpTpGpG. Therefore, the assignment of the H_8 resonances in d-GpG can be extended to d-TpGpG, d-TpTpGpG, and d-CpTpTpGpG. These assignments of H_8 resonances in d-TpTpGpG and d-CpTpTpGpG are reliable because the thymine base has a very weak ring-current effect and thus has little shielding effect on the neighboring guanine base. Additional evidence may be obtained from the ^1H NMR study of d-TpG, d-TpGpG, and d-GpG. The chemical shift value of G-H_8 in d-TpG is 8.05 ppm which occurs 0.19 ppm downfield from that of G^9-H_8 in d-TpGpG. The chemical shift value of G^9-H_8 in d-GpG (7.84 ppm) differs from that in d-TpGpG by only 0.02 ppm This result strongly suggests that the G^2-H_8 proton can be effectively shielded by its 3′ neighbor (G) but not by its 5′ neighbor (T) in d-TpGpG. Similar observations were made in the assignment of H_2 and H_8 resonances in d-CpCpA and d-CpCpApA as discussed in the previous section.

H_6 and Methyl Group of Thymine. The signals of H_6 and CH_3 can be easily distinguished not only by their different intensities but also by their chemical shift values. The assignment of H_6 and CH_3 of thymine in d-TpGpG is straightforward. In d-TpTpGpG and d-CpTpTpGpG, the H_6 resonance at higher field, closer to that in d-TpGpG, is assigned to T^8-H_6 and the one at lower field is assigned to T^7-H_6. This assignment is reasonable because T^8 in d-TpGpG, d-TpTpGpG, and d-CpTpTpGpG is shielded mainly by G^9, whereas T^7 is shielded mainly by T^8. Guanine is known to exhibit a stronger ring-current shielding effect than thymine (Giessner-Prettre et al., 1976). Therefore, the T^8-H_6 should resonate at a higher field position than the H_6 of T^7. However, the chemical shift values of the two CH_3 signals in d-TpTpGpG and d-CpTpTpGpG are very close ($\simeq$0.01 ppm apart). A similar situation is also observed at the low temperature (Figure 2). The exact assignment of these two CH_3 signals is difficult, but at present not important.

The assignments of the base proton resonances of d-CpCpApApG and d-CpTpTpGpG (as well as their smaller fragments) were made at high temperature. The assignments at low temperature can be easily made by tracing the temperature profiles, as shown in Figure 2. The crossing over in the graphs (Figure 2a,c) can be identified by following the lines closely. Therefore, the complete assignment of base proton resonances in these oligonucleotides is based on (i) the distinct characteristics of individual base proton resonances, (ii) the various NMR measurements (such as T_1 measurement decoupling, etc.), and (iii) the information from smaller fragments, i.e., the sequence incremental method (Borer et al., 1975). These techniques are also very useful for the assignment of sugar proton resonances of short nucleic acids, as described in the following section.

(b) Assignments and the Measurements of Chemical Shifts and Coupling Constants of Deoxyribofuranose Proton Reso-

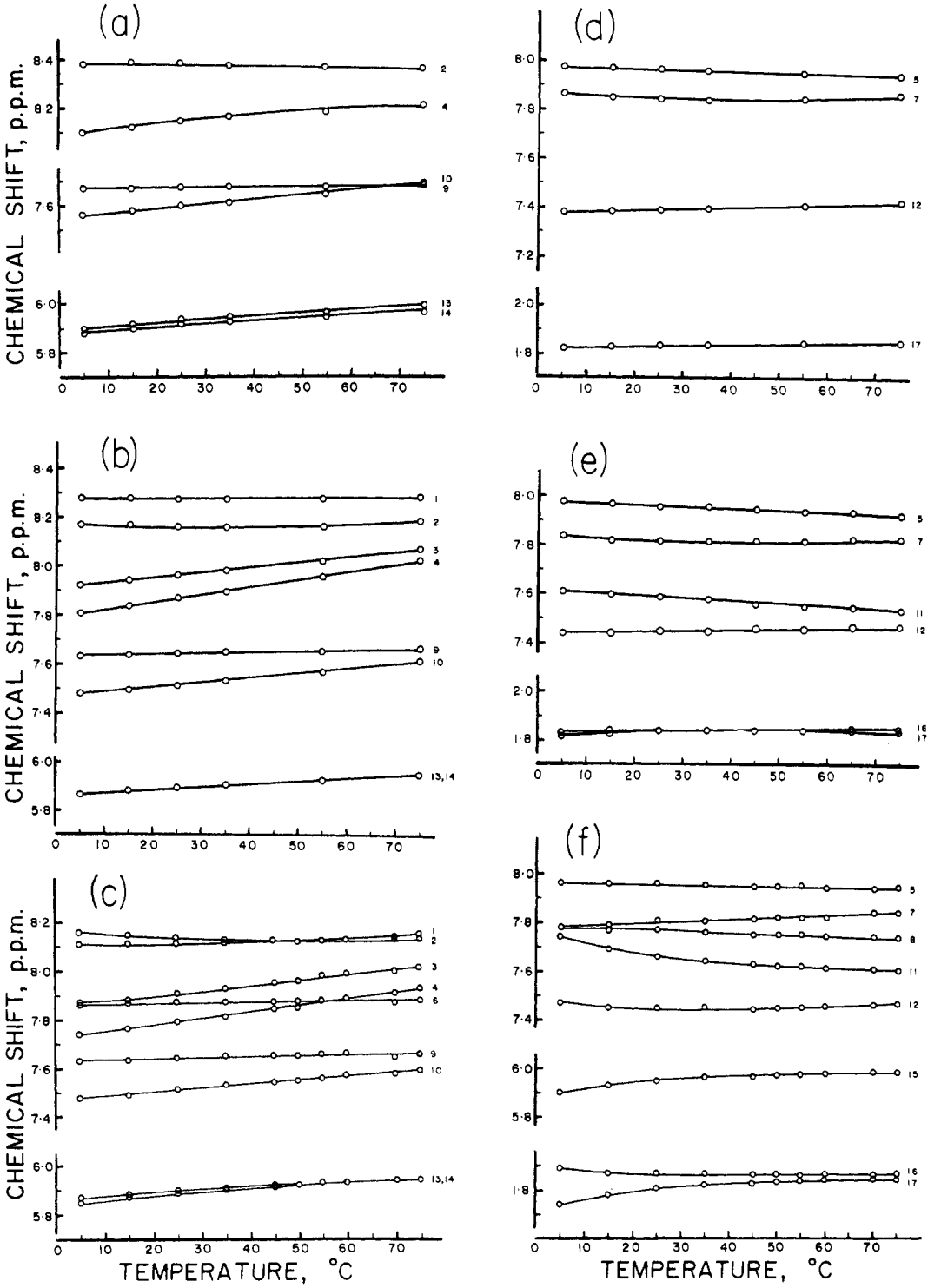

FIGURE 2: Chemical shifts vs. temperature profiles of base proton resonances of d-CpCpA (a), d-CpCpApA (b), d-CpCpApApG (c), d-TpGpG (d), d-TpTpGpG (e), and d-CpTpTpGpG (f). The numbers represent the following: 1 = A^4-H_8, 2 = A^3-H_8, 3 = A^4-H_2, 4 = A^3-H_2, 5 = G^{10}-H_8, 6 = G^5-H_8, 7 = G^9-H_8, 8 = C^6-H_6, 9 = C^1-H_6, 10 = C^2-H_6, 11 = T^7-H_6, 12 = T^8-H_6, 13 = C^1-H_5, 14 = C^2-H_5, 15 = C^6-H_5, 16 = T^7-CH_3, and 17 = T^8-CH_3.

nances of d-CpCpApApG, d-CpTpTpGpG, and Their Smaller Fragments. The ^1H NMR spectra of the sugar proton resonances of these oligonucleotides have two features. First, the backbones are composed of deoxyribose. Therefore, only seven kinds of sugar proton resonances need to be considered. However, each type of proton resonance has very similar chemical shifts. Second, the sugar protons are coupled with their neighboring proton(s) or phosphorus, but they are not coupled with the proton(s) of other sugar units. Therefore, all proton resonances belonging to the same sugar unit can be grouped by sequential decoupling techniques (Cheng & Sarma, 1977) before actual identity of this sugar moiety is revealed. In other words, all resonances from one sugar unit can be assigned if one of them is determined. In this paper, the incremental assignment method (Borer et al., 1975) has been applied to the $H_{2'}$ resonances since the $H_{1'}$ region of d-CpTpTpGpG is extensively overlapped. As mentioned in the previous section, these two pentamers represent two different situations depending on the direction of the growth of the oligonucleotide chain in the incremental assignment procedure.

In d-CpTpTpGpG, the chain grows from the 3′ to the 5′, and the added bases are pyrimidines. Therefore the ring-

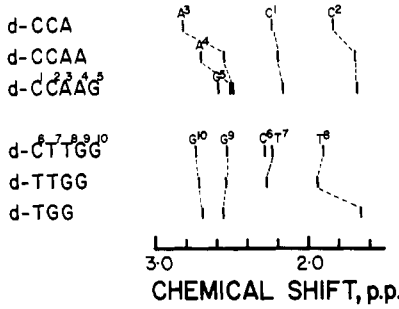

FIGURE 3: Incremental assignment scheme of $H_{2'}$ resonances of deoxytrimers, -tetramers, and -pentamers at 20–25 °C.

current shielding effect from the newly added base(s) to the existing oligomer is minimal. Thus the assignment of the $H_{2'}$ resonances in this pentamer is straightforward (Figure 3). As shown in Figure 3, the $H_{2'}$ resonances of T^8, G^9, and G^{10} can be unambiguously assigned. However, for C^6 and T^7, two $H_{2'}$ resonances are only 0.05 ppm apart. Therefore, additional investigation is necessary. From d-TpTpGpG to d-CpTpTpGpG, the $H_{2'}$ resonance of T^8 is moved 0.04 ppm upfield as the result of added C^6 to the tetramer (Figure 3). Therefore, it is reasonable for the $H_{2'}$ resonance of T^7 in d-CpTpTpGpG to be shifted upfield by 0.04 ppm due to the addition of C^6 to the tetramer. By comparing, the spectral pattern of d-TpTpGpG and d-CpTpTpGpG, the extra signals can be assigned to $H_{2'}$ and $H_{2''}$ of C^6 with confidence (Figures 5 and 6b). Moreover, such assignments were confirmed by sequential decoupling techniques starting with the $H_{5'}$, and $H_{5''}$. The $H_{5'}$ and $H_{5''}$ resonances of the 3′ moiety (C^6 in d-CpTpTpGpG) can be readily distinguished from $H_{5'}$ and $H_{5''}$ resonances of other 5′ moieties due to the lack of ^{31}P coupling constants.

In d-CpCpApApG, the chain length grows from 5′ to 3′, and the additional nucleotides are purine base(s). Therefore the ring-current shielding effect has to be considered. In Figure 3, the $H_{2'}$ resonances of C^1 and C^2 in this pentamer can be readily assigned from trimer because these two residues are relatively distant from the addition site of the chain elongation. Therefore, the chemical shifts of the $H_{2'}$ are not greatly affected by the added purines. As shown in Figure 3, the $H_{2'}$ resonance of C^2 is moved 0.14 ppm upfield from d-CpCpA to d-CpCpApA, and the upfield shift becomes even less from tetramer to d-CpCpApApG. In the case of $H_{2'}$ of C^1, the upfield shift is very small from trimer to pentamer. In d-CpCpApA, the A^3-$H_{2'}$ resonance is shifted upfield by A^4. Therefore, it resonates at a higher field than A^4-$H_{2'}$. Similarly, the A^3-$H_{2'}$ and A^4-$H_{2'}$ resonances in d-CpCpApApG are shifted upfield by G^5 (Figure 3). As shown in Figure 3, $H_{2'}$ resonances of A^3 and A^4 are only 0.01 ppm apart in the pentamer; therefore such assignments are tentative and may be reversible. However, through the homodecoupling approach described below, the whole set ($H_{1'}$, $H_{2'}$, $H_{2''}$, $H_{3'}$, $H_{4'}$, $H_{5'}$, and $H_{5''}$) of resonances of each pentose unit has been identified. The current assignment of $H_{2'}$(s) of A^3 and A^4 leads to the assignment of $H_{1'}$, $H_{4'}$, $H_{5'}$, and $H_{5''}$ of A^3 located about 0.07–0.01 ppm upfield than those of A^4 residues. This result is consistent with the expectation of the ring-current effect of A vs. G as well as the residue at the midposition should be more shielded than the residue nearer to the terminus. It should be noted further that if the A^3-$H_{2'}$ and A^4-$H_{2''}$ assignments are indeed reversed, our conclusions on the conformation of the backbone remain uneffected (see Table V), since these two sets of residue protons have very similar coupling constants. From Cheng and Sarma's data, the $H_{2'}$ resonance of pdG resonates at 2.81 ppm while the $H_{2'}$ resonance of -pdG in d-ApG resonates at a lower field compared to $H_{2'}$ of dAp- (2.75 ppm vs. 2.34 ppm). Therefore, it is reasonable to assign G^5-$H_{2'}$ in d-CpCpApApG at the lower field than the A^3-$H_{2'}$ and A^4-$H_{2'}$ (2.58 ppm vs. 2.50 and 2.49 ppm).

After the $H_{2'}$ resonances in these two pentamers are identified, then the other sugar proton resonances can be assigned through the decoupling technique. The spectral pattern of $H_{5'}$ and $H_{5''}$ of the Np- moiety is distinctly different from those of other residues due to the lack of the phosphorus couplings. These two proton signals always resonate at the highest field as compared to the other $H_{5'}$ and $H_{5''}$ resonances (Figures 4–6). These $H_{5'}$ and $H_{5''}$ resonances can be assigned by comparing their spectral pattern and chemical shift values with their dimeric fragments. The approach can be used to confirm the previous assignment.

Finally, it should be mentioned that there is no direct method to distinguish the $H_{5'}$ and $H_{5''}$ resonances. The signal at lower field is tentatively assigned to $H_{5'}$ (Lee et al., 1976). In addition, the resonances from $H_{2'}$ and $H_{2''}$ can be distinguished by their coupling constants with $H_{1'}$, namely, $J_{1'2'}$ (trans) is larger than $J_{1'2''}$ (cis) (Davies & Danyluk, 1975).

The chemical shifts and coupling constants of all sugar proton resonances of d-CpCpApApG and d-CpTpTpGpG as well as their smaller fragments are summarized in Tables I–IV. Data for the two pentamers are given only at 20 °C, at which the 600-MHz spectra were obtained.

(*c*) *Applicability of the "Sequential Incremental Assignment" Method.* The sequential incremental method was first applied to a hexanucleotide, r-ApApGpCpUpU (Borer et al., 1975). The fragments used were r-ApA, r-ApApG, r-ApApGpC, and r-ApApGpCpU. Therefore, growth of the fragment chains is in the 5′ to 3′ direction, and the added nucleosides were guanosine, cytosine, and uracil bases (they

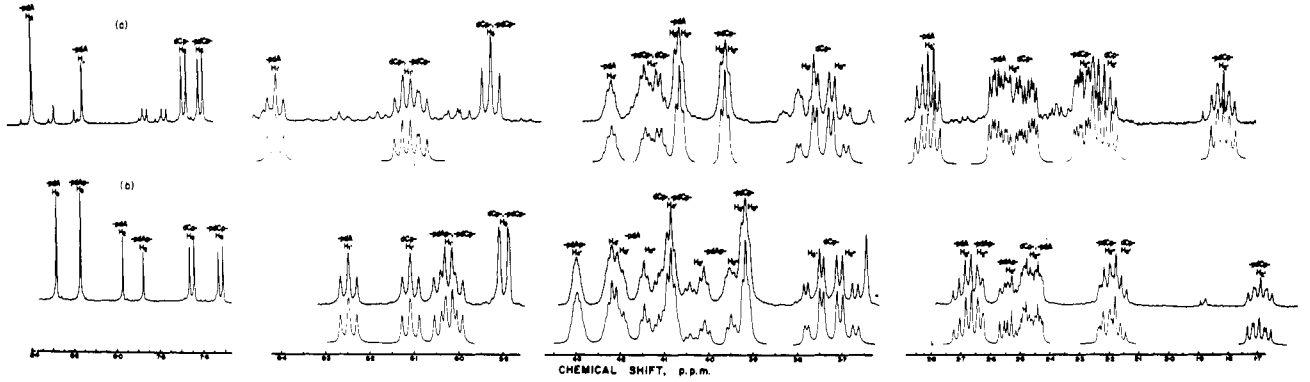

FIGURE 4: 360-MHz ^1H NMR spectra of d-CpCpA (111 OD) (a) and d-CpCpApA (178 OD) (b) at 25 °C (100 scans). The line-shape simulations based on values listed in Tables I and III are at the bottom of the observed spectra.

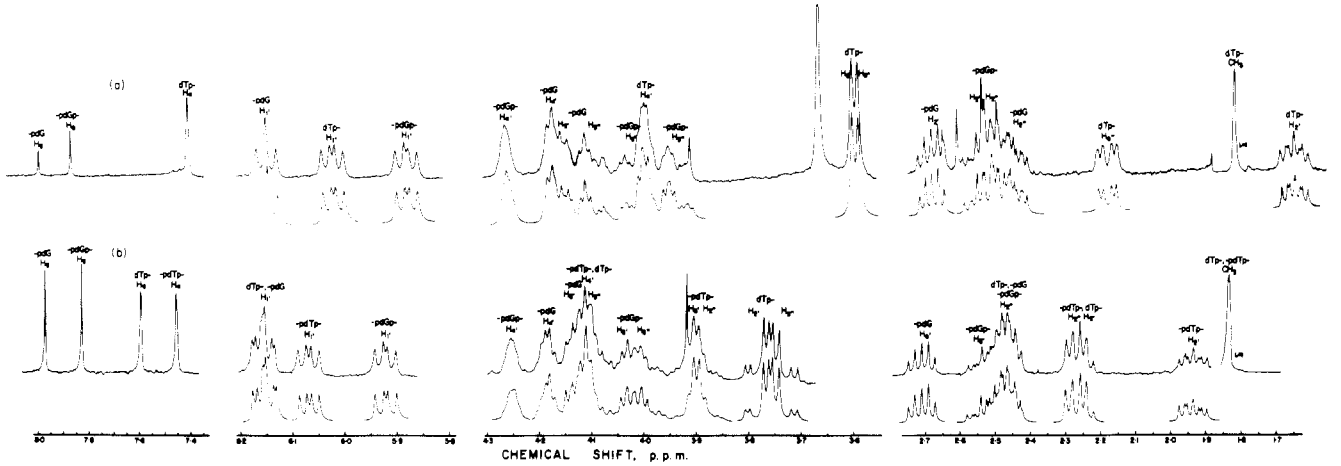

FIGURE 5: 360-MHz ^{1}H NMR spectra of d-TpGpG (84 OD) (a) and TpTpGpG (113 OD) (b) at 25 °C (200 scans). The line-shape simulations based on values listed in Tables II and IV are at the bottom of the observed spectra.

Table I: Chemical Shifts (ppm) from DSS of d-CpCpA, d-CpCpApA, and d-CpCpApApG in D_2O

compds		temp (°C)	$H_{1'}$	$H_{2'}$	$H_{2''}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$
d-$C^1C^2A^3$	C^1	25	6.12	2.23	2.49	4.72	4.12	3.78	3.72
	C^2		6.09	1.83	2.29	4.68	4.15	3.97	3.96
	A^3		6.41	2.81	2.58	4.73	4.22	4.08	4.07
d-$C^1C^2A^3$	C^1	75	6.17	2.25	2.52	4.74	4.13	3.79	3.72
	C^2		6.16	1.99	2.41	4.69	4.20	4.01	3.98
	A^3		6.44	2.80	2.59	4.71	4.24	4.08	4.07
d-$C^1C^2A^3A^4$	C^1	25	6.10	2.19	2.46	4.68	4.09	3.76	3.69
	C^2		6.00	1.69	2.21	4.64	4.09	3.92	3.91
	A^3		6.03	2.54	2.66	4.93	4.30	4.02	3.94
	A^4		6.24	2.69	2.47	4.75	4.22	4.21	4.13
d-$C^1C^2A^3A^4$	C^1	75	6.15	2.23	2.50	4.71	4.10	3.77	3.70
	C^2		6.09	1.87	2.33	4.68	4.13	3.97	3.92
	A^3		6.18	2.57	2.63	4.92	4.30	4.02	3.99
	A^4		6.35	2.74	2.54	4.72	4.24	4.17	4.12
d-$C^1C^2A^3A^4G^5$	C^1	20	6.10	2.16	2.45	4.63	4.08	3.75	3.68
	C^2		6.00	1.68	2.19	4.67	4.10	3.91	3.90
	A^3		5.87	2.49	2.59	*a*	4.26	4.00	3.91
	A^4		5.96	2.50	2.59	*a*	4.33	4.10	4.10
	G^5		5.96	2.58	2.35	4.67	4.13	4.19	4.08

a Under HDO.

have weaker ring current than adenine). On the basis of the information from these fragments, the base proton and $H_{1'}$ resonances can be readily assigned (Borer et al., 1975). In this paper, we would like to test rigorously the applicability of this method to oligonucleotides with other sequences. The sequences of two deoxypentanucleotides studied with their fragments were carefully designed. As mentioned in the previous section, d-CpCpApApG is growing from the 5′ to 3′ end and is the same as those in r-ApApGpCpUpU, except the added nucleotides are carrying adenine and guanine bases (these bases exert a much stronger ring-current effect than cytosine and thymine). The d-CpTpTpGpG oligomer is growing from the 3′ to 5′ end and the added bases are cytosine and thymine. In summary, the results in sections a and b as well as the earlier work from our laboratory (Borer et al., 1975) indicate that in the cases of r-ApApGpCpUpU and d-CpTpTpGpG, the assignment of all nonexchangeable proton resonances can be accomplished by this incremental method directly. This result is due to the small ring-current anisotropic shielding effect exerted by the "incrementally added" bases in chain elongation of the fragment. This "null" effect can be attributed to either the weak ring-current effect of the added bases or the relatively long distance of the newly added base at the 5′ end; both factors result in minimal shielding effect exerted by the newly added bases to the base(s) originally in the fragment. In the case of d-CpCpApApG where purine bases are added to the growing fragment, the incremental procedure can also be applied with an additional assumption. In this case, the shielding effect of adenine or guanine bases

Table II: Chemical Shifts (ppm) from DSS of d-TpGpG, d-TpTpGpG, and d-CpTpTpGpG in D_2O

compds		temp (°C)	$H_{1'}$	$H_{2'}$	$H_{2''}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$
d-$T^8G^9G^{10}$	T^8	25	6.02	1.66	2.19	4.60	4.00	3.60	3.60
	G^9		5.88	2.56	2.50	4.92	4.26	4.02	3.94
	G^{10}		6.14	2.69	2.45	4.70	4.18	4.16	4.10
d-$T^8G^9G^{10}$	T^8	75	6.06	1.94	2.32	4.64	4.01	3.66	3.62
	G^9		6.04	2.61	2.54	4.91	~4.34	~4.02	~4.00
	G^{10}		6.19	2.73	2.48	4.69	*a*	~4.13	~4.10
d-$T^7T^8G^9G^{10}$	T^7	25	6.16	2.27	2.48	*a*	4.10	3.78	3.73
	T^8		6.07	1.94	2.27	*a*	4.11	3.91	3.88
	G^9		5.92	2.54	2.46	4.89	4.25	4.04	3.99
	G^{10}		6.15	2.71	2.47	*a*	4.19	4.15	4.10
d-$T^7T^8G^9G^{10}$	T^7	75	6.15	2.29	2.48	4.73	4.11	3.77	3.72
	T^8		6.10	2.05	2.35	4.74	4.14	3.95	3.93
	G^9		6.04	2.60	2.51	4.89	*a*	4.03	4.01
	G^{10}		6.18	2.73	2.47	4.68	4.18	4.11	4.08
d-$C^6T^7T^8G^9G^{10}$	C^6	20	6.15	2.28	2.54	~4.66	4.17	3.82	3.76
	T^7		6.23	2.23	2.45	~4.74	4.28	4.10	4.04
	T^8		5.98	1.90	2.24	~4.66	4.05	3.93	3.89
	G^9		5.89	2.53	2.47	~4.79	4.23	3.99	3.97
	G^{10}		6.15	2.73	2.48	~4.66	4.19	4.16	4.09

a Under HDO.

Table III: Coupling Constants [J (Hz)] of d-CpCpA, d-CpCpApA, and d-CpCpApApG in D_2O

compds		temp (°C)	$J_{H_1'-H_2'}$	$J_{H_1'-H_2''}$	$J_{H_2'-H_2''}$	$J_{H_2'-H_3'}$	$J_{H_2''-H_3'}$	$J_{H_3'-H_4'}$	$J_{H_4'-H_5'}$	$J_{H_4'-H_5''}$	$J_{H_5'-H_5''}$	$J_{H_3'-P}$	$J_{H_4'-P}$	$J_{H_5'-P}$	$J_{H_5''-P}$
d-$C^1C^2A^3$	C^1	25	6.9	6.3	−13.9	6.4	3.5	3.4	3.0	4.2	−12.4	7.0			
	C^2		7.7	6.1	−14.0	6.2	2.7	2.5	2.5	3.6	−11.2	7.0	3.5	2.5	3.6
	A^3		6.8	6.6	−14.0	6.8	3.9	3.5	2.5	3.6	−11.2		2.5	2.5	3.6
d-$C^1C^2A^3$	C^1	75	6.9	6.5	−14.2	6.6	3.6	3.8	3.7	4.7	−12.4	6.7			
	C^2		7.6	5.9	−14.0	6.6	2.9	3.0	2.7	4.2	−11.7	7.0	2.0	4.5	4.6
	A^3		7.0	6.4	−14.0	6.5	3.9	3.7	3.0	3.9	−11.2		2.4	4.0	5.0
d-$C^1C^2A^3A^4$	C^1	25	7.3	6.3	−14.0	6.3	3.7	3.0	3.5	4.4	−12.4	6.7			
	C^2		8.0	5.0	−14.6	5.6	2.2	3.1	2.4	3.6	−11.8	6.6	3.5	2.4	3.6
	A^3		8.9	5.6	−14.0	5.4	1.5	3.1	3.3	3.0	−11.8	6.3	2.0	4.3	3.7
	A^4		7.3	6.2	−14.0	6.8	3.8	3.0	2.7	2.7	−11.3		3.2	5.0	3.2
d-$C^1C^2A^3A^4$	C^1	75	7.0	6.5	−14.0	6.7	3.9	3.4	3.6	4.9	−12.6	6.7			
	C^2		8.0	5.9	−14.0	6.6	2.4	3.9	3.1	4.0	−11.6	6.7	3.6	5.3	4.4
	A^3		8.6	6.3	−14.4	6.0	2.3	3.0	4.0	3.5	−11.6	6.4	2.0	4.3	5.0
	A^4		7.0	6.5	−14.0	6.7	3.9	3.0	2.8	3.9	−11.6		3.2	4.7	4.9
d-$C^1C^2A^3A^4G^5$	C^1	20	7.2	6.2	−14.0	6.8	3.5	3.4	3.6	4.6	−12.4	6.7			
	C^2		8.6	5.8	−14.0	6.6	2.1	2.8	2.0	3.2	−11.6	6.7	1.8	3.2	3.2
	A^3		9.4	5.7	−13.9	5.5	1.7	2.8	3.2	2.5	−11.6	*a*	1.7	3.8	2.6
	A^4		9.4	5.5	−13.9	5.5	1.7	2.6	2.8	3.0	−11.4	*a*	1.7	2.8	3.0
	G^5		7.4	6.7	−13.9	6.8	4.2	2.8	3.0	2.0	−11.4		2.0	4.2	4.0

[a] Too close to HDO.

Table IV: Coupling Constants [J (Hz)] of d-TpGpG, d-TpTpGpG, and d-CpTpTpGpG in D_2O

compds		temp (°C)	$J_{H_1'-H_2'}$	$J_{H_1'-H_2''}$	$J_{H_2'-H_2''}$	$J_{H_2'-H_3'}$	$J_{H_2''-H_3'}$	$J_{H_3'-H_4'}$	$J_{H_4'-H_5'}$	$J_{H_4'-H_5'}$	$J_{H_5'-H_5''}$	$J_{H_3'-P}$	$J_{H_4'-P}$	$J_{H_5'-P}$	$J_{H_5''-P}$
d-$T^8G^9G^{10}$	T^8	25	8.8	5.3	−14.0	5.9	1.0	3.2	4.2	4.2	−12.6	6.5			
	G^9		8.9	5.5	−14.0	6.7	1.8	2.8	2.9	3.1	−12.7	6.5	1.9	4.3	4.7
	G^{10}		6.7	6.4	−14.0	6.5	4.4	3.6	2.8	2.6	−11.8		2.0	3.5	5.2
d-$T^8G^9G^{10}$	T^8	75	7.7	6.0	−14.3	6.4	2.8	3.2	3.9	4.8	−12.2	6.5			
	G^9		7.8	5.5	−14.0	5.6	2.5	3.0	~3.2	−3.2	−12.0	6.5		~4.5	~4.5
	G^{10}		7.3	6.4	−14.0	6.8	4.0	4.0							
d-$T^7T^8G^9G^{10}$	T^7	25	7.6	6.8	−14.0	7.1	2.7	3.0	3.4	4.6	−12.5	*a*			
	T^8		8.3	5.4	−14.6	5.5	1.4	3.0	3.5	3.7	−12.2	*a*	2.0	4.0	4.4
	G^9		8.5	6.2	−14.2	5.6	2.0	3.1	3.5	3.6	−12.0	6.4	1.6	4.4	4.8
	G^{10}		7.0	6.2	−14.0	6.5	4.0	3.4	3.0	3.7	−11.6		2.0	4.6	4.4
d-$T^7T^8G^9G^{10}$	T^7	75	7.6	6.3	−14.4	7.4	3.3	3.3	3.4	4.9	−12.2	6.6			
	T^8		7.8	5.3	−14.2	5.8	2.0	3.4	2.8	3.7	−11.6	6.7	1.9	2.8	3.7
	G^9		8.3	6.1	−14.2	5.8	2.4	3.4	3.7	3.7	−11.4	6.7	*a*	4.8	4.8
	G^{10}		7.1	6.2	−14.0	6.6	4.2	3.4	3.4	3.8	−11.4		2.0	4.8	5.0
d-$C^6T^7T^8G^9G^{10}$	C^6	20	7.0	5.8	−13.8	7.0	3.6	3.6	3.1	4.6	−12.7	*b*			
	T^7		8.4	5.8	−13.5	6.8	1.9	2.4	3.0	3.0	−11.4	*b*	1.6	4.0	3.2
	T^8		9.1	6.0	−14.0	6.2	1.9	2.6	3.2	3.0	−11.4	*b*	1.6	4.0	4.6
	G^9		8.7	6.5	−13.7	5.3	2.7	2.8	3.6	3.7	−11.4	*b*	1.8	3.9	4.4
	G^{10}		7.0	6.4	−14.0	6.8	4.5	2.8	2.5	3.5	−11.4		1.7	3.8	3.8

[a] Under HDO. [b] Too close to HDO.

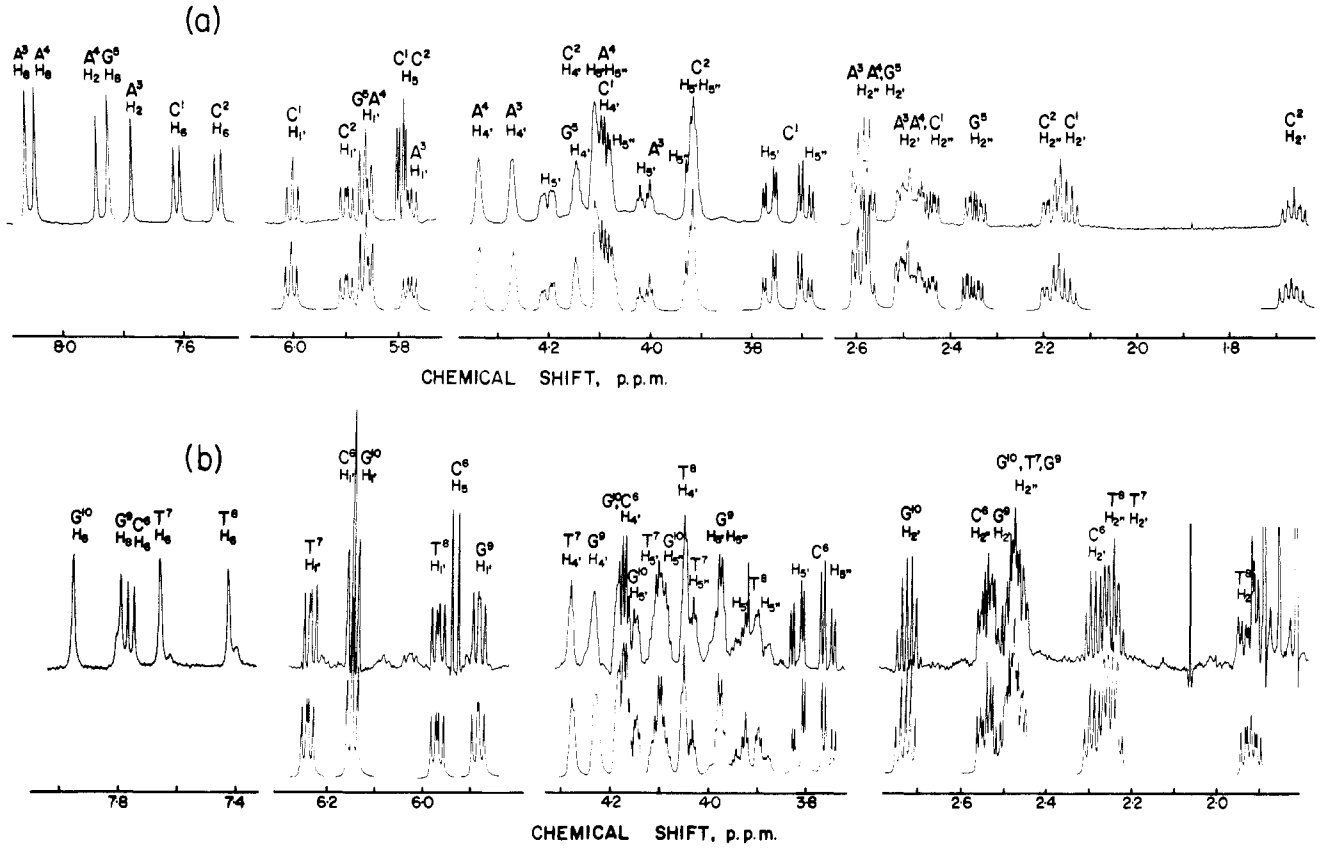

FIGURE 6: 600-MHz ^{1}H NMR spectra of d-CpCpApApG (174 OD) (a) and d-CpTpTpGpG (152 OD) (b) at 20 °C (100 scans). The line-shape simulations based on values listed in Tables I–IV are at the bottom of the observed spectra. The extra peaks beside the T-CH_3 peaks in (b) are due to the spike from the computer, because such peaks did not show in either 500- or 360-MHz ^{1}H NMR spectra of the same compound.

is being considered with the assumptions that the turn of the oligonucleotide is right-handed and the nucleosidyl units are in anti form. The reasoning of the assignment of all nonexchangeable proton resonances of d-CpCpApApG has been presented above. Thus, this "incremental" method has been shown to be successful for the assignment of all nonexchangeable proton resonances in these three oligonucleotides (two pentamers and one hexamer) of very different base composition and sequences.

(*d*) *Base-Stacking Conformation of d-CpCpApApG, d-CpTpTpGpG, and Their Short Fragments.* The profiles of chemical shift values of base proton resonances of all six deoxyoligonucleotides vs. temperature were shown in Figure 2. Three conclusions can be drawn: (i) The profiles of chemical shift vs. temperature can serve as extra pieces of evidence for the assignments. For example, the two H_6 of C^1 and C^2 in d-CpCpA are very close to each other (Figure 2a). They can be still assigned by their different response to the changing temperature. (ii) The major ring-current anisotropic shielding effect exerted on the base and sugar protons of any residue in the oligonucleotide is from its 3′-end neighbor(s). For example, the base proton resonances of A^3 are shifted upfield by A^4, and those of A^4 are not being shielded upfield by A^3 to the same extent. Finally, (iii) all six deoxyoligonucleotides in aqueous solution are obviously in single-stranded forms. This is indicated by the monotonic profiles observed in the chemical shift vs. temperature plot (Figure 2), in contrast to those observed for the helix–coil transition (Borer et al., 1975; Miller et al., 1980).

(*e*) *Conformation of the Deoxyribofuranose Ring.* The conformation of the deoxyribose ring in nucleotides has been discussed in detail by Cheng & Sarma (1977). The deoxyribose ring exists predominantly as an equilibrium blend of ^2E- and ^3E-type conformations. For computation of the population of ^2E conformers, the value for $J_{1'2'}$ and $J_{3'4'}$ was obtained by averaging the trimers, tetramers, or pentamers at both low and high temperatures. These values for the deoxytrimers, -tetramers, and -pentamers are 10.8, 11.0, and 11.1 Hz, respectively. When the sum of $J_{1'2'}$ and $J_{3'4'}$ and the observed magnitude of $J_{1'2'}$ are employed, the percentage population of ^2E-type conformers in the deoxytrimers, -tetramers, and -pentamers was computed and is listed in Table V. The value of $J_{1'2'}$ is used in computation instead of $J_{3'4'}$ because the coupling constant value $J_{1'2'}$ can be determined more precisely than $J_{3'4'}$ in these complicated spectra. Data in Table V indicate that at 25 °C these deoxytrimers, -tetramers, and -pentamers show a clear preference to populate in ^2E-type conformations, and only a minimal reduction in the ^2E population was observed when the temperature was elevated to 75 °C.

These deoxytrimers, -tetramers, and -pentamers of different bases, therefore, provide an opportunity to study the effects of chain length, the nature of base(s), and the direction of the chain growth on the sugar conformation. A careful analysis of the data shown in Table VI as well as the results of the dideoxynucleoside monophosphates from Cheng & Sarma (1977) reveal that the percentage of ^2E population of oligonucleotides depends on the existence of another nucleotide at the 3′ end as well as the nature of the base in the nucleotide. For instance, the percentage of the ^2E conformer of dCp is 66 and increases to 72 when a 5′-deoxyadenylic acid is added on its 3′-OH end (i.e., d-CpA). However, the percentage of ^2E conformer remains unchanged (66% and 67%) when another 5′-deoxycytodylic acid or a 5′-thymidylic acid is added on the 3′-OH end of pdC as in d-CpC and d-CpT (Cheng & Sarma, 1977). The effect on increasing ^2E population by the

Table V: Population Distribution of Conformers in Deoxytrimers, -tetramers, and -pentamers[a]

		temp	d-$C^1C^2A^3A^4G^5$				d-$C^1C^2A^3A^4$				d-$C^1C^2A^3$			
		(°C)	% ^2E	% gg	% $g'g'$	ϕ'	% ^2E	% gg	% $g'g'$	ϕ'	% ^2E	% gg	% $g'g'$	ϕ'
d-$C^1C^2A^3A^4G^5$	C^1	25	65	57		199°/281°	66	60		199°/281°	64	67		200°/280°
	C^2		77	88	89	199°/281°	73	79	91	199°/281°	71	78	91	200°/280°
	A^3		85	82	89		81	76	82	198°/282°	63	78	91	
	A^4		85	81	92		66	86	81					
	G^5		67	90	81									
	C^1	75					64	54		199°/281°	64	55		199°/281°
	C^2						73	68	74	199°/281°	70	70	76	200°/280°
	A^3						78	64	75	198°/282°	65	70	77	
	A^4						64	72	74					
	G^5													

		temp	d-$C^6T^7T^8G^9G^{10}$				d-$T^7T^8G^9G^{10}$				d-$T^8G^9G^{10}$			
		(°C)	% ^2E	% gg	% $g'g'$	ϕ'	% ^2E	% gg	% $g'g'$	ϕ'	% ^2E	% gg	% $g'g'$	ϕ'
d-$C^6T^7T^8G^9G^{10}$	C^6	25	63	62										
	T^7		76	79	86	199°/281°	69	59						
	T^8		82	77	79	199°/281°	75	67	80		81	55		198°/282°
	G^9		78	66	80		77	68	76	198°/282°	82	79	77	198°/282°
	G^{10}		63	79	84		64	72	77		62	86	78	
	C^6	75												
	T^7						69	56		199°/281°				
	T^8						71	74	89	199°/281°	71	52		198°/282°
	G^9						75	65	74	199°/281°	72	~75	~77	198°/282°
	G^{10}						65	67	73		68			

[a] % $^2E = [J_{1'2'}/(J_{1'2'} + J_{3'4'})] \times 100$; accurate to ±3–4%. % $gg = [(13.7 - \Sigma)/9.7] \times 100$; $\Sigma = J_{4'5'} + J_{4'5''}$; accurate to ±5–6%. % $g'g' = [(25 - \Sigma')/20.8] \times 100$; $\Sigma' = J_{5'P} + J_{5''P}$; accurate to ±5–6%. $^3J_{Hp} = 18.1 \cos^2 \theta_{Hp} - 4.8 \cos \theta_{Hp}$; $\phi' = 240° \pm \theta$; accurate to ±3°. The two deoxypentamers were measured at 20 °C only.

Table VI: Comparison of Population Distribution of Conformers in Deoxymonomers, -dimers, -trimers, -tetramers, and -pentamers at 20–25 °C

	% ^2E					% gg					% $g'g'$				
	C^1	C^2	A^3	A^4	G^5	C^1	C^2	A^3	A^4	G^5	C^1	C^2	A^3	A^4	G^5
dCp	66					58									
pdC		65					70					75			
d-C^1C^2	66	68				67	75					76			
d-$C^1C^2A^3$	64	71	63			67	78	78				91	91		
d-$C^1C^2A^3A^4$	66	73	81	66		60	79	76	86			91	82	81	
d-$C^1C^2A^3A^4G^5$	65	77	85	85	67	57	88	82	81	90		89	89	92	81

	% ^2E					% gg					% $g'g'$				
	C^6	T^7	T^8	G^9	G^{10}	C^6	T^7	T^8	G^9	G^{10}	C^6	T^7	T^8	G^9	G^{10}
dGp				76					57						
pdG					70					63					67
d-G^9G^{10}				65	60				57	90					86
d-$T^8G^9G^{10}$			81	82	62			55	79	86				77	78
d-$T^7T^8G^9G^{10}$		69	75	77	64		59	67	68	72			80	76	77
d-$C^6T^7T^8G^9G^{10}$	63	76	82	78	63	62	79	77	66	79		86	79	80	84

addition of a 5′-deoxyadenylic acid to the 3′-OH end becomes more clear as the chain length increases. The ^2E percent of A^3 in d-CpCpA is 63 and then increased to 81 in d-CpCpApA as a result of the addition of A^4 on the 3′-OH end of A^3 (Table VI). Addition of 5′-deoxyguanosine has a similar effect as the addition of 5′-deoxyadenylic acid. For example, the ^2E percent of A^4 nucleoside in d-CpCpApA is 66 and increases to 85 in d-CpCpApApG. A similar conclusion can be drawn from the d-CpTpTpGpG series. For example, the ^2E percent of T^8 deoxypentose in d-TpGpG is 81 and is much higher than that in Tp (67) and pT (68). There are many observations in Table VI and in the literature (Cheng & Sarma, 1977) to support the following conclusions: (i) No effect of the 3′-nucleotidyl unit can be observed on the ^2E population of the 5′-nucleosidyl unit. This is why the ^2E percent values of the free 3′-end nucleoside of all six oligonucleotides are very low and close to those of the monomers in spite of the nature of the base. (ii) The addition of a 5′-nucleotidyl unit to the 3′-OH end exerts a significant effect on increasing the ^2E population of the 3′-unit, but (iii) the magnitude of such an effect depends on the nature of the base added on the 3′ end, i.e., A, G ≫ C, T. (iv) This effect of adding a 5′-nucleotidyl unit to the 3′ end is greatest in the nearest 3′-nucleoside and is reduced significantly on the 3′-nucleoside one unit away (next nearest neighbor); i.e., the ^2E percent of C^2 is almost the same (or increased slightly) in d-CpCpA (71), d-CpCpApA (73), and d-CpCpApApG (77).

(*f*) *Backbone Conformations.* The population distribution of conformers about the $C_{4'}$–$C_{5'}$ (ψ) and $C_{5'}$–$O_{5'}$ (ϕ) bonds of nucleotides and dioligonucleotides have been well discussed (Lee & Sarma, 1975, 1976; Kan et al., 1980). The computed populations of *gg* and *g′g′* for deoxytrimers, -tetramers, and -pentamers are compiled in Table V. The data show that there is a general preference for *gg* and *g′g′* conformers for d-CpCpA, d-TpGpG, d-CpCpApA, d-TpTpGpG, d-CpCpApApG, and d-CpTpTpGpG. Elevation of temperature causes a reduction in *gg* and *g′g′* populations, with the effect being more pronounced in the cases of d-CpCpApA and d-CpCpA.

As shown in Table VI, the values of percent *gg* of free 3′ ends are much higher than those at free 5′ ends in the d-CpCpApApG series [75% (3′) vs. 67% (5′) in d-CpC; 78% (3′) vs. 67% (5′) in d-CpCpA; 86% (3′) vs. 60% (5′) in d-CpCpApA; 90% (3′) vs. 57% (5′) in d-CpCpApApG]. This observation indicates that the rotation of the $C_{4'}$–$C_{5'}$ bond at the 5′ ends is less restricted than that at the 3′ ends, and the torsion angle of this $C_{4'}$–$C_{5'}$ bond at the 3′ ends is predominantly in the *gg* form. The same result is found in the d-CpTpTpGpG series (Table VI). As for the rotation of the $C_{5'}$–$O_{5'}$ bond, the population of *g′g′* is very high in both d-CpCpApApG and d-CpTpTpGpG series (Table VI). This result indicates that the torsion angle of $C_{5'}$–$O_{5'}$ bond exists predominantly at $\phi = 180°$.

The method for calculating the rotamer distribution about the $C_{3'}$–$O_{3'}$ bond (ϕ') has been extensively discussed elsewhere (Cheng & Sarma, 1977; Kan et al., 1980). Data from Table V shows that deoxytrimers, -tetramers, and -pentamers have similar ϕ' values, i.e., $\simeq 200°/280°$, which can be designated as the ϕ'_-/ϕ' domains for the $C_{3'}$–$O_{3'}$ torsion. It has been shown that detectable quantities of ${}^2E\phi'_+$ species will be manifested in the four-bond coupling $J_{H_{2'}-P}$ (Lee & Sarma, 1975). No such four-bond couplings were observed in these short oligomers. Hence, it appears that the torsion about the $C_{3'}$–$O_{3'}$ bond is restricted to a domain around $\phi' \simeq 200°$. Neither temperature nor increase in chain length from trimer, tetramer, to pentamer has any effect on the average torsion angle of this $C_{3'}$–$C_{3'}$ bond.

Conclusion

The achievement in the complete assignments of all non-exchangeable protons of these deoxytrimers, -tetramers, and -pentamers paves the way for assignment of all the protons of the helical duplexes of d-CpCpApA + d-TpTpGpG and d-CpCpApApG + d-CpTpTpGpG as well as assignment of the ^{13}C resonances and ^{31}P resonances of these single-stranded short oligomers by the heterodecoupling technique.

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Supplementary Material Available

Preparation and purification of the oligodeoxyribonucleotides (11 pages). Ordering information is given on any current masthead page.

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