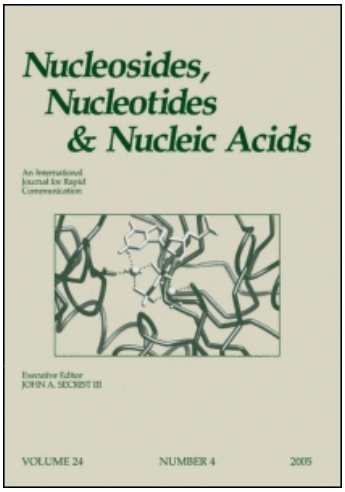

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New Sequential-Assignment Routes of Nucleic Acid NMR Signals Using a [5′-^{13}C]-Labeled DNA Dodecamer†

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ABSTRACT

NMR signal assignments for DNA oligomers have been performed by the well-established sequential assignment procedures based on NOESY and COSY. The H4′/H5′/H5″ resonance region is congested and difficult to analyze without the use of isotope-labeled DNA oligomers. Here a DNA dodecamer constructed with 2′-deoxy[5′-^{13}C]ribonucleotides, 5′-d(*C*G*C*G*A*A*T*T*C*G*CG)-3′ (*N = [5′-^{13}C]Nucleotide), was prepared in an effort to analyze the H4′/H5′/H5″ resonance region by 2D ^{1}H-^{13}C HMQC-NOESY. In the C5′ and H1′ resonance region, weak and strong cross peaks for C5′(i)-H1′(i) and C5′(i)-H1′(i-1), respectively, were found, thus enabling the sequential assignment within this region. A similar sequential assignment route was found between C5′ and H2″. Proton pair distances evaluated from the canonical B-DNA as well as A-DNA indicated that these

†In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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sequential-assignment routes on a 2D ^{1}H-^{13}C HMQC-NOESY spectrum work for most nucleic acid stem regions.

Key Words: NMR; [5′-^{13}C]DNA; C5′ resonance assignment; C5′-H1′ sequential assignment; C5′-H2″ sequential assignment; 2D ^{1}H-^{13}C HMQC-NOESY.

INTRODUCTION

An investigation of the conformational diversity of the sugar-phosphate backbone and/or the sugar moieties in nucleic acids is important in the elucidation of nucleic acid-protein or -drug recognition processes. The development of an efficient synthetic method for 2′-deoxy[5′-^{2}H]ribonucleosides (5′*S*:5′*R* = ca. 2:1)[1,2] has fueled expectations that they would facilitate the unambiguous assignment of both the H5′ and H5″ signals of an oligodeoxyribonucleotide and the analysis of sugar-phosphodiester backbone conformations by NMR spectroscopy. Through the use of 2D ^{1}H-^{31}P HSQC spectroscopy on deuterium-labeled nucleotides, Ono and co-workers[3] achieved the assignment of the methylene protons at C5′ of a DNA dodecamer. On the other hand, Kojima and co-workers[4] obtained the 15 ^{3}J coupling constants between H4′ and H5′/H5″ due to the simplified spin systems through the use of NOESY and DQF-COSY NMR analyses. We assumed that more precise analyses of the DNA backbone structure including distance information should be possible by using nucleotides labeled with carbon-13 at the 5′ position, in terms of heteronuclear multidimensional NMR spectroscopy. Conformational analysis by NMR using a [5′-^{13}C]DNA-oligonucleotide, however, has never been reported. Thus, following the development of an efficient method for the synthesis of 2′-deoxy[5′-^{13}C]ribonucleosides,[5,6] we now report on the construction of a [5′-^{13}C]DNA-oligonucleotide and its conformational analysis by HMQC-NOESY NMR. In relation to the C5′ resonance assignment, we found several new sequential-assignment routes on a 2D ^{1}H-^{13}C HMQC-NOESY spectrum. Details of the results obtained are described herein.

RESULTS AND DISCUSSION

The 2′-deoxy[5′-^{13}C]nucleosides[5,6] were converted into the corresponding 3′-phosphoramidite derivatives according to the method of Ono and co-workers.[7–9] These were used for the construction of 5′-d(*^{1}C*^{2}G*^{3}C*^{4}G*^{5}A*^{6}A*^{7}T*^{8}T*^{9}C*^{10}G*^{11}C^{12}G)-3′ by the solid-phase phosphoramidite method[10] using G-CPG at the 3′ terminus. The residue number used here is shown on the left shoulder. Purification of the oligonucleotide was performed according to the method of Kyogoku and co-workers.[11]

All ^{1}H and ^{31}P assignments for this oligomer at 30°C have been reported by Hare and co-workers,[12] Kellogg and Schweitzer,[13] and Ono and co-workers.[3] For the assignment of each C5′ resonance, the reported ^{1}H chemical shifts[3,12,13] provided useful information. Through the use of the ^{1}H-^{13}C HSQC spectrum combined with the ^{1}H assignments, the 5′-^{13}C signals were easily assigned for 7 of the 11 residues: C1, T7, C9, C3, A6, G4 and A5 (Figure 1).

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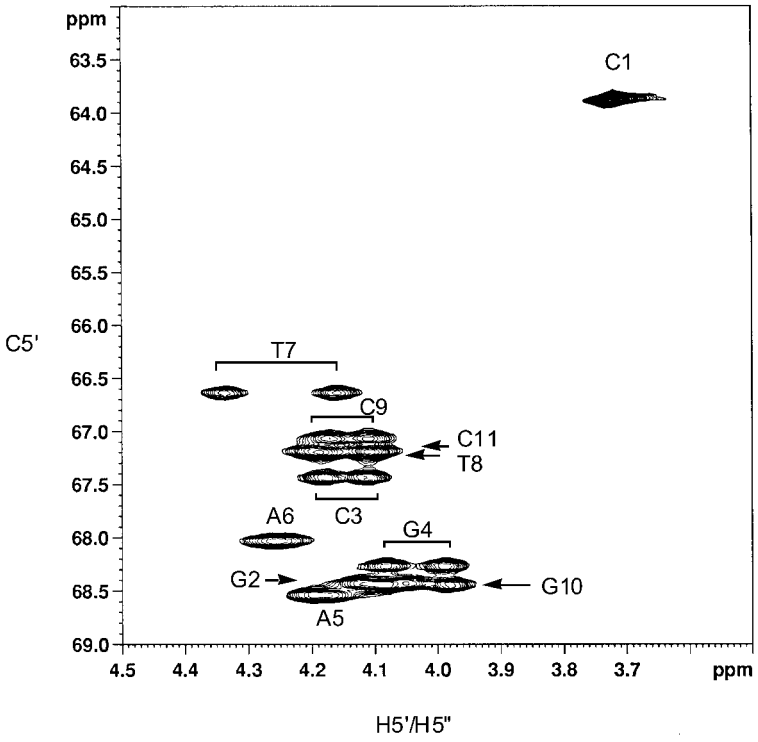

Figure 1. C5′–H5′ spectral region of the ^{1}H-^{13}C HSQC spectrum of the [5′-^{13}C]-labeled DNA dodecamer [5′-d(*^{1}C*^{2}G*^{3}C*^{4}G*^{5}A*^{6}A*^{7}T*^{8}T*^{9}C*^{10}G*^{11}C^{12}G)-3′]. Resonance assignments are given by residue names.

Another spectrum was required to complete the assignments due to the overlapping of the ^{1}H and ^{13}C signals. In Figure 2, the C5′ (w1) and H1′ (w2) region of the ^{1}H-^{13}C HMQC-NOESY spectrum is shown. In this region, the C5′-H1′ sequential connectivity was found, as indicated by the sequential-walk line between the inter-residue H1′(i-1)-C5′(i) (strong) and intra-residue H1′(i)-C5′(i) cross-peaks (weak). Application of this sequential-walk enabled all of the 5′-^{13}C signals to be unambiguously assigned: 64.0 (C1), 66.7 (T7), 67.2 (C9), 67.3 (T8 and C11, overlap), 67.5 (C3), 68.1 (A6), 68.4 (G4), 68.5 (G2 and G10, overlap), and 68.6 (A5), ppm (residue name), respectively. The assignment of each H1′ was also achieved using the sequential-walk line between the inter-residue H1′(i-1)-C5′(i) (strong) and intra-residue H1′(i)-C5′(i) cross-peaks (weak) (Figure 2).

A similar sequential connectivity was found between the C5′ and H2″ signals, although the intensity of the cross peak was much weaker than that of C5′-H1′. Based on the 5′-^{13}C assignments, several intra-residue NOE cross-peaks were assigned: H6/8(i)-C5′(i), H2′(i)-C5′(i) and H3′(i)-C5′(i). In particular, the H6/8(i)-C5′(i) region of the ^{1}H-^{13}C HMQC-NOESY spectrum was sparse, and thus H6 and H8 were easily assigned. The inter- and/or intra-residue H4′-C5′ NOE cross-peaks were also observed, but were not completely assigned due to signal overlapping. These intra-residue NOEs

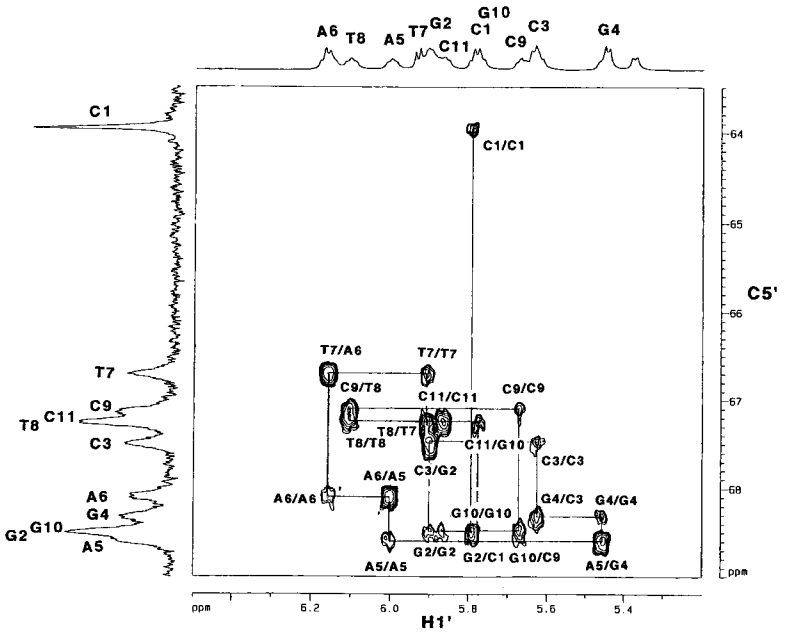

Figure 2. C5′–H1′ spectral region of the 2D ^{1}H-^{13}C HMQC-NOESY spectrum of the [5′-^{13}C]-labeled DNA dodecamer. Resonance assignments are given by the residue names. Lines in the spectrum indicate the sequential assignment routes.

were used to confirm the sequential assignments made by the H1′-C5′ and H2″-C5′ cross-peaks.

The H1′-C5′ and H2″-C5′ sequential-walks found here were analyzed by examining proton-pair distances including H4′/H5′/H5″ as shown in Tables 1 and 2 for canonical B- and A-DNA, respectively. The intra- and inter-residue H1′–H5′, H2″–H5′, H2″–H5″, H3′–H5′, H3′–H5″, H4′–H5′ and H4′–H5″ distances were always shorter than 5 Å. That is, NOEs can be observed for these pairs in both the B- and A-DNA conformations (see Tables 1 and 2). Thus 7 sequential assignment routes were potentially available in the NOESY spectrum. Since the H5′ and H5″ signals could not be distinguished in our 2D HMQC-NOESY, 3 out of 7 routes were degenerate, while 4 routes were available. In fact, two of these, C5′–H1′ and C5′–H2″, were found in our HMQC-NOESY spectrum, though the other two, C5′–H3′ and C5′–H4′, could not identified in our spectrum due to signal overlapping. New sequential assignment routes seem to be powerful for nucleic acids with either the A or B conformation. NOESY-based sequential assignments have been employed even for ^{13}C-enriched DNA and RNA oligomers. This is because sequential assignment through small ^{13}C–^{31}P *J*-couplings does not work well in some cases. Therefore, the NOESY-based sequential assignment routes found here are quite important.

The H4′/H5′/H5″ region (3.9 ∼ 4.4 ppm, ^{1}H) in the NOESY spectrum contains ample structural information, as previously pointed out by Wijmenga and co-workers[14] and Kojima and co-workers.[4] Selective ^{13}C enrichment at the 5′ methylene

Table 1. Distances of the proton pairs including H4′/H5′/H5″ below 5 Å in the canonical B-DNA conformation.[a]

	H8/H6	H5	M[#]	H1′	H2′	H2″	H3′	H4′	H5′	H5″
Intra-residue										
H4′ (i)	4.9/4.6	–	–	3.6	3.9	4.1	2.7	†	2.6	2.3
H5′ (i)	3.5/3.3	5.0	∼ 5	4.4	3.7	4.9	3.7	2.6	†	1.8
H5″ (i)	4.4/4.2	–	–	–	3.9	5.0	2.9	2.3	1.8	†
Inter-residue										
H4′ (i + 1)[b]	–/–	–	–	4.2	–	–	–	–	–	–
H4′ (i-1)[b]	–/–	–	–	–	–	–	–	–	3.8	4.1
H5′ (i + 1)[b]	–/–	–	–	1.7	4.3	3.2	4.5	3.8	–	–
H5′ (i-1)[b]	–/–	–	–	–	–	–	–	–	–	–
H5″ (i + 1)[b]	–/–	–	–	3.3	–	4.0	4.7	4.1	–	–
H5″ (i-1)[b]	–/–	–	–	–	–	–	–	–	–	–

[a]Distances in Å.
[b]Inter-residue proton pair has two combinations for each residue, that is, (i-1)th residue to (i)th residue and (i)th to (t + 1)th. (i) refers to the focusing residue. (i-1) and (i + 1) refer to the former and later rows, respectively.
[#]: Methyl proton.
–: Distance longer than 5 Å.
†: Identical proton.

Table 2. Distances of the proton pairs including H4′/H5′/H5″ below 5 Å in the canonical A-DNA conformation.[a]

	H8/H6	H5	M[#]	H1′	H2′	H2″	H3′	H4′	H5′	H5″
Intra-residue										
H4′ (i)	4.3/4.0	–	–	3.3	3.8	2.8	3.0	†	2.5	2.4
H5′ (i)	3.6/3.3	–	–	4.6	–	4.9	3.7	2.5	†	1.8
H5″ (i)	4.2/3.9	–	–	–	–	4.7	3.1	2.4	1.8	†
Inter-residue										
H4′ (i + 1)[b]	–/–	–	–	–	4.3	4.0	–	–	–	–
H4′ (i-1)[b]	–/–	–	–	–	–	–	–	–	4.0	5.0
H5′ (i + 1)[b]	–/–	–	–	3.6	2.7	1.6	4.3	4.0	–	–
H5′ (i-1)[b]	–/–	–	–	–	–	–	–	–	–	–
H5″ (i + 1)[b]	–/–	–	–	–	3.7	3.1	4.8	5.0	–	–
H5″ (i-1)[b]	–/–	–	–	–	–	–	–	–	–	–

[a]Distances in Å.
[b]Inter-residue proton pair has two combinations for each residue, that is, (i-1)th residue to (i)th residue and (i)th to (t + 1)th. (i) refers to the focusing residue. (i-1) and (i + 1) refer to the former and later rows, respectively.
[#]: Methyl proton.
–: Distance longer than 5 Å.
†: Identical proton.

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position makes it possible to distinguish the H5′/5″ signals from others, especially from H4′. The unambiguous assignment of the heavily overlapped H4′/H5′/H5″ signals has been the main impediment in the complete assignment of the NOESY cross-peaks for DNA. Therefore, it is important that the 5′-^{13}C enriched DNA sample completely overcomes this overlapping problem. Additionally, the use of two ^{13}C-edited NOESY spectra, where the H5′/5″ signals are selectively picked up or eliminated, can yield the complete NOESY data sets without any overlapping between the H4′ and H5′/5″ resonances.

Using a uniformly ^{13}C-enriched sample, it would not be difficult to distinguish the H5′/5″ signals from those of H4′, since the ^{13}C chemical shifts are different for C4′ (80 $\sim$ 85 ppm) and C5′ (60 $\sim$ 65 ppm). However, the transverse relaxation time of the 5′-^{13}C signals is much shorter than the other carbons, thus the constant-time approach, that can refocus the ^{13}C–^{13}C *J*-couplings, does not work well. As a result, the apparent linewidths of the 5′-^{13}C signals for the uniformly ^{13}C-enriched sample will be much broader, and the new sequential assignment routes reported here might not work well.

CONCLUSION

New sequential assignment routes using 5′-^{13}C signals, C5′–H1′ and C5′–H2″, were found in the 2D ^{1}H-^{13}C HMQC-NOESY spectrum. Using these new routes, all of the 5′-^{13}C and the H1′/H2″ signals were completely and easily assigned. The salient feature of these assignment routes was the use of the 5′-^{13}C signals, rather than H5′ and H5″, to reduce the complexity of the NOESY spectrum. These sequential assignment routes will become a powerful tool in the second or third sequential-walk routes for most nucleic acids, and in the assignment of the H4′/H5′/H5″ signals.

EXPERIMENTAL SECTION

Sample preparation. The 2′-deoxy[5′-^{13}C]nucleoside derivatives (N^6-benzoyl-2′-deoxyadenosine, N^2-acetyl-O^6-diphenylcarbamoyl-2′-deoxyguanosine, N^4-benzoyl-2′-deoxycytidine, and thymidine), reported by Kawashima and co-workers,[5,6] were converted into the corresponding 3′-phosphoramidite derivatives according to the method of Ono and co-workers.[7–9] 5′-d(*^{1}C*^{2}G*^{3}C*^{4}G*^{5}A*^{6}A*^{7}T*^{8}T*^{9}C*^{10}G*^{11}C^{12}G)-3′ was then synthesized on a DNA synthesizer (Applied Biosystems Inc., ABI 391) by the solid-phase phosphoramidite method[10] using G-CPG (purchased from Applied Biosystems, Inc.) at the 3′ terminus. The [5′-^{13}C]DNA-oligonucleotide was subsequently purified after the removal of the protecting group. The purification procedure employed was that previously reported by Kyogoku and co-workers.[11] The residue number used here is shown on the left shoulder. The NMR sample was dissolved in D_2O and kept in a 5 mm tube. The double-strand concentration was estimated to be 1.5 mM from the UV absorbance.

NMR experiments. The two dimensional ^{1}H-^{13}C proton-detected heteronuclear single-quantum correlation spectroscopy (HSQC) spectrum and the ^{1}H-^{13}C HMQC-NOESY[15] spectrum were recorded on a Bruker DMX 600 spectrometer operating at a

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600 MHz ^{1}H frequency at 30°C, where the spectral widths were 2000 and 6000 Hz with 256 and 2048 recording points in the ^{13}C (t1) and ^{1}H (t2) dimensions, respectively. The π/4 shifted sine bell window function was applied and zero-filled to 1024 (^{13}C) and 4096 (^{1}H) points. The ^{13}C-edited $^{1}H-^{1}H$ NOESY by Otting and coworkers[16] was recorded with identical parameters except for the t1 dimension, recorded at 6000 Hz with 512 recording points at the ^{1}H frequency. The pulse repetition delay time was 2 sec, and $80 \sim 112$ scans were employed for each t1 increment. The phase sensitive detection in t1 was performed by the TPPI procedure reported by Marion and Wüthrich.[17] The ^{1}H and ^{13}C chemical shifts were calculated relative to the resonance of solvent H_2O (4.68 ppm) for HMQC[18] and from TSP (3-(trimethylsilyl)propionic acid) for the HMQC and NOESY spectra.

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