cases, A_∞ could be calculated by use of a known weight of reactant or determined by catalyzing the reaction to completion by addition of a small amount of hydroxide ion. Amine buffer solutions used ranged from 0.05 to 0.40 M. Reaction progress was monitored in a scanning mode or at 247 nm.

Reaction rates for the pseudo-first-order reactions were determined by using a least-squares computer plotting program written in SBASIC for the Dartmouth Time-Sharing System. Use of the program required the input of A_∞ and absorbances with their respective times as determined experimentally. The observed reaction rate, k_{obsd}, is given by the least-squares slope of the line determined by plotting ln $(A_\infty - A_{time})$ versus time.

For a series of reactions at differing buffer concentrations but at a constant pH, the hydroxide-catalyzed rate of reaction, $k_{OH}[OH^-]$, was obtained by extrapolation to zero-buffer concentration, and division by $[OH^-]$ gave the value of k_{OH}. The rate of the amine-catalyzed reaction was obtained by subtracting $k_{OH}[OH^-]$ from k_{obsd}. The concentrations of free and protonated amine, [AM] and $[AMH^+]$, were calculated from the expressions [AM] = [total buffer] $\times k_a/(k_a + a_H)$ and $[AMH^+]$ = [total buffer] − [AM]. Values of k_B were calculated from $k_B = (k_{obsd} - k_{OH}[OH^-])/[AM]$.

With morpholine as a catalyst, the contribution of the $k_{AB}[AM][AMH^+]$ term was evaluated by using the expression $(k_{obsd} - k_{OH}[OH^-])/[AM] = k_{AB}[AMH^+] + k_B$ and iteratively fitting data to it, starting with an appropriate value of k_B determined where $k_B[AM]$ accounts for >90% of k_{obsd}.

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Registry No. **4**, 66875-62-5; **7**, 114200-69-0; **8**, 114200-76-9; **9**, 114200-80-5; **10**, 114200-72-5; **12**, 20721-86-2; **13**, 114200-65-6; **14**, 114200-66-7; **15**, 114200-67-8; **16**, 114200-68-9; **17**, 114200-70-3; **18**, 1196-55-0; **19**, 72938-41-1; **20**, 114200-73-6; **21**, 114200-77-0; **22**, 114200-74-7; **23**, 114200-75-8; **24**, 114200-78-1; **25**, 114200-79-2; **26**, 64281-61-4; **27**, 114200-81-6; 1α-deuterio-9β-hydroxy-*cis*-decal-2-one, 114200-71-4; morpholine, 110-91-8; *N*-methylmorpholine, 109-02-4; *N*-ethylmorpholine, 100-74-3; deuterium, 7782-39-0.

Selective Deuteriation as an Aid in the Assignment of ^1H NMR Spectra of Single-Stranded Oligodeoxynucleotides

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Abstract: The thymidine C5 methyl and C6 proton resonances in the ^1H NMR spectrum of the tetradeoxynucleotide TpTpTpT were assigned with the aid of selective deuteriation. Hydrogen was replaced with deuterium in the methyl group of thymidine by platinum-catalyzed exchange in D_2O. The trideuteriothymidine was converted to the protected mononucleotide for either solution- or solid-phase phosphotriester oligodeoxynucleotide synthesis chemistry. Two tetradeoxynucleotides TpTpTpT were prepared with defined percentages of [*methyl*-$^1H_3$]thymidine and [*methyl*-$^2H_3$]thymidine at each position in the sequence. Assignment of the methyl signals was accomplished by integration and confirmed by examination of the aromatic region of the spectrum. The thymine H6 protons were assigned by homonuclear decoupling experiments and inspection of the ^1H NMR spectrum to determine the relative ratio of each resonance that appeared as a quartet and as a singlet as a result of the isotopic substitution at the adjacent methyl group. The catalyzed exchange of deuterium for hydrogen in the thymidine methyl group was found to proceed efficiently with a modest amount of catalyst. The partial deuteriation method described in this paper should be useful for the assignment of single-stranded oligodeoxynucleotides, duplexes in which B-DNA geometry is not followed, and novel oligodeoxynucleotide structures.

Much attention has been directed recently to the ^1H NMR spectral assignment of Watson–Crick oligodeoxynucleotide duplexes by use of two-dimensional nuclear Overhauser effect techniques (NOESY).[1a-f] These methods assume that the oligodeoxynucleotide is in the B-DNA conformation and rely on the nucleotides being held in relatively constrained positions so that dipolar coupling may be observed between protons on the heterocyclic bases, the corresponding deoxyribose moiety, and the neighboring 5′-deoxyribose. The connectivity between bases and the neighboring 5′ deoxyribose sugars may be followed through the sequence and the assignments deduced.

Less attention has been paid to the spectral assignment of single-stranded oligomers and duplex oligomers containing nucleotide sequences not in the Watson–Crick conformation. In these situations, there is often insufficient conformational stability to observe specific intra- and internucleotide NOEs necessary to derive sequence information. The two-dimensional nuclear Overhauser effect experiment, which gives good results when applied to a Watson–Crick duplex, often fails with a single-stranded oligomer.

Borer et al.[2] developed and used the method of incremental analysis to assign the ^1H NMR resonances of oligonucleotides. They synthesized a series of short oligomers and analyzed the NMR spectrum of each at high temperature. When the spectrum of the smaller oligomer from the next larger in the series was compared, the resonances of the added nucleotide were assigned. Recently, several promising NMR methods for the sequence assignment of single-stranded oligonucleotides have been devel-

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oped.[3a-e] These methods, which do not depend on prior knowledge or assumptions about molecular conformation, utilize single- and multiple-quantum heteronuclear $^{31}P-^{1}H$ correlation spectroscopy and ^{31}P-relayed $^{1}H-^{1}H$ correlation spectroscopy to establish the connectivities between nucleotide units in the chain. The relayed experiment is particularly exciting because it allows sequential connectivities to be made between H3′ of a particular nucleotide and H4′ of the neighbor 3′-nucleotide without resolving the ^{31}P spectrum, which is often difficult. Even so, the H4′ resonances must be resolvable, and the coherence-transfer experiment must be repeated several times in order to optimize for a range of coupling constants.[3b]

A potential solution to part of the assignment problem might be found in selective partial deuteriation. Sequence assignments could be determined in a single experiment if an oligomer were synthesized with different defined isotopic ratios at a single site at each individual position in the sequence. Such an approach has been previously utilized for assignment of ^{31}P spectra of oligonucleotides through synthetic $^{17}O/^{18}O$ labeling procedures.[4a-d] In this paper we demonstrate the method by comparing the ^{1}H spectra of two different TpTpTpT's which have been synthesized with defined ratios of CH_3 and CD_3 in each thymidyl unit. The thymidine methyl signals are then assigned on the basis of area of the ^{1}H signals. The CD_3-containing thymidine was synthesized by a simple procedure[5] and converted to the 5′-*O*-(4,4′-dimethoxytrityl)thymidine 3′-*O*-(*o*-chlorophenyl phosphate) for phosphotriester synthesis using standard methods.[6a]

Experimental Section

Materials and Methods. Thymidine, triethylammonium 5′-*O*-(4,4′-dimethoxytrityl)thymidine 3′-*O*-(*o*-chlorophenyl phosphate), 4,4′-dimethoxytrityl chloride, 3′-*O*-benzoylthymidine, and 4-chlorophenyl phosphodichloridate were obtained from commercial sources. Mesitylenesulfonyl 3-nitro-1,2,4-triazolide, protected trideuteriothymidine mononucleotides, and the tetradeoxynucleotides were prepared by standard phosphotriester methodology,[6a-d] with minor modification.

^{1}H NMR spectra were standardized internally to DSS (D_2O) or TMS ($CDCl_3$). ^{2}H spectra in H_2O were standardized to HDO and those in $CHCl_3$ or CH_2Cl_2 to $CDCl_3$. ^{13}C spectra in D_2O were standardized to the methyl group of acetonitrile and those in $CDCl_3$ to $CDCl_3$. ^{1}H spectra of the tetradeoxynucleotides were acquired at 400 MHz at 40 °C in 0.4 mL of 99.96% D_2O buffered with 0.01 M sodium phosphate, pH 7.4, 5×10^{-5} M EDTA, and 0.1 M sodium chloride. Acquisitions were zero-filled to 32K data points before Fourier transformation. Resolution enhancement by Gaussian multiplication was employed to improve observation of closely spaced multiplets. The extinction coefficient (260 nm) of 3.3×10^4 M^{-1} cm^{-1} was calculated for TpTpTpT by using tabulated monomer and dimer extinction coefficients.[7]

2′-Deoxy-5-(trideuteriomethyl)uridine (Trideuteriothymidine). The nucleoside containing deuterium was prepared by a modification of the platinum-catalyzed exchange method of Kinoshita et al.[5] (see the Discussion). Thymidine (6 g, 2.5 mmol) was dissolved in 30 mL of 2:1 D_2O/pyridine, and the solution was evaporated. The residue was dissolved in a minimum of hot D_2O, the solution evaporated, and the residue redissolved in 75 mL of D_2O in a 250-mL Parr bottle. The solution was degassed by alternate freezing and thawing in vacuo. Platinum catalyst, freshly prepared by hydrogenolysis of 0.5 g (2.0 mmol) of PtO_2 in D_2O, was added to the bottle, which was then sealed with a rubber septum. The bottle was filled with hydrogen gas through a syringe needle and the mixture allowed to equilibrate for 1 h. The bottle was evacuated briefly, and 12 mL (~5 μmol) of hydrogen was added. The mixture was stirred and heated at 50 °C for 3 days. The addition of hydrogen was repeated; after another 2 days, the incorporation was 40%. The mixture was filtered through powdered cellulose, the solvent was evaporated, and fresh catalyst (from 0.3 g of PtO_2) and D_2O were added. The bottle was filled with hydrogen, and the mixture was heated at 60 °C for an additional 3 days. The platinum was then removed by filtration, and the solution was evaporated to about 25 mL, at which point 3.0 g of product crystallized from the water. Continued evaporation and crystallization yielded a total of 4.5 g (75%) of trideuteriothymidine: mp 184–186 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.66 (s, 1, H_6), 6.28 (apparent t, 1, $H_{1'}$, J = 6.8 Hz), 4.47 (m, 1, $H_{4'}$), 4.02 (m, 1, $H_{3'}$), 3.85 (dd, 1, $H_{5'}$, J = 12.4, 3.6 Hz), 3.77 (dd, 1, $H_{5''}$, J = 12.4, 5.1 Hz), 2.37 (m, 2, $H_{2',2''}$), 1.85 (br s, 0.17, residual ^{1}H on the 5-CH_3 group) (The methyl signal represents a mixture of CD_2H, CDH_2, and CH_3 species. Deuterium incorporation was 94.2%, as determined by the ratio of the residual methyl signal and the signal for $H_{1'}$; ^{13}C NMR (100 MHz, D_2O) δ 167.12 (2-C=O), 152.37 (4-C=O), 138.26 (C_6), 112.02 (C_5), 87.32 ($C_{4'}$), 85.89 ($C_{1'}$), 71.26 ($C_{3'}$), 62.03 ($C_{5'}$), 39.34 ($C_{2'}$), 11.58 (m, CD_3) (In a comparison of the ^{13}C spectrum of thymidine-d_3 with that of undeuteriated thymidine, C5 was shifted upfield 0.12 ppm. The CD_3 multiplet was of greatly reduced intensity and shifted upfield by 0.6 ppm); ^{2}H NMR (61.4 MHz, H_2O, 40 °C) δ 1.85 (CD_3), no exchange visible at any other position; MS (positive ion FAB) m/e 246 ($M + H^+$), 228 ($M + H^+ - H_2O$), 130 [*methyl*-$^{2}H_3$]thymine + H^+); UV (water) ϵ_{max} 9.33×10^3 at 267 nm.

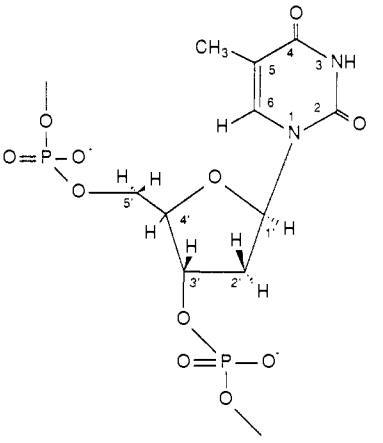

Figure 1. Structure and numbering of thymidine. For the TpTpTpT tetradeoxynucleotide, T_1 refers to the 5′-end nucleotide, T_2 to the second, T_3 to the third, and T_4 to the 3′-end.

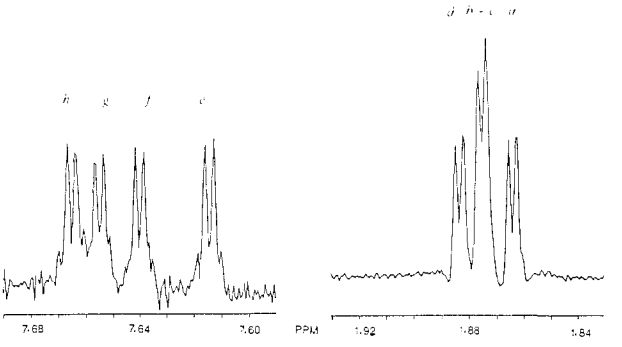

Figure 2. Expansion of the aromatic (left) and methyl (right) regions of the 400-MHz spectrum of TpTpTpT in which all the thymines have CH_3. Gaussian multiplication of the FID was used to enhance resolution of the lines.

Preparation of Specifically Deuteriated TpTpTpT (25, 50, 75, 100% CH_3) by Phosphotriester Solid-Phase Chemistry. The tetramer containing [*methyl*-$^{1}H_3$]thymine (percentages: T_1 25; T_2, 50; T_3, 75; T_4, 100) was prepared and purified as above on a 4-μmol scale; T_1 refers to the 5′-end nucleotide, T_2 to the second, T_3 to the third, and T_4 to the 3′-end. The protected mononucleotide for the first addition (T_3) was made by the mixture of 4.2 mg (6 μmol) of the protected deuteriated monomer and 12.5 mg (18 μmol) of the protected nondeuteriated monomer. The mixture for T_2 contained 8.4 mg (12 μmol) of each monomer and that for T_1 contained 12.6 mg (18 μmol) of the deuteriated and 4.2

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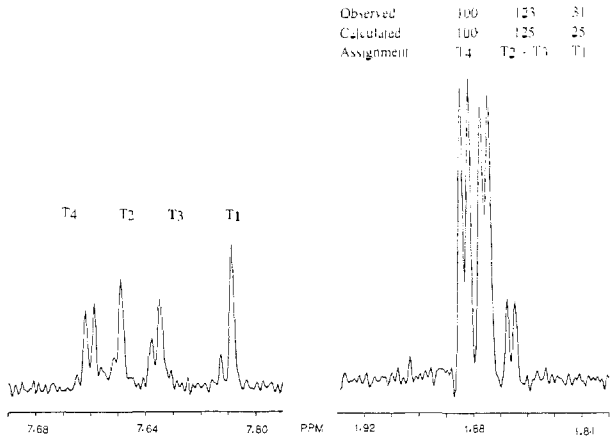

Figure 3. Expansion of the aromatic and methyl regions of the spectrum of TpTpTpT in which the thymines have CH_3 in the ratio of 25:50:75:100 in T_1, T_2, T_3, and T_4, respectively.

mg (6 μmol) of the nondeuteriated monomers. Tetramer: yield 18.4 A_{260} units (14% overall, A_{260}/A_{280} = 1.40).

Preparation of Specifically Deuteriated TpTpTpT (40, 53, 66, 100% CH_3) by Phosphotriester Solution Chemistry. The tetramer containing [*methyl*-$^1H_3$]thymine (percentages: T_1, 40; T_2, 53; T_3, 66; T_4, 100) was prepared by phosphotriester solution chemistry. The deuteriated and undeuteriated protected mononucleotides were mixed in large batches to minimize inaccuracies resulting from errors during the weighing of the components. The ratios of CH_3 to CD_3 were checked by NMR. Comparison of the integral of the methyl signal with the two methoxyl groups and the anomeric proton indicated the percentages of CH_3 to be 40, 53, and 66 in the three CH_3/CD_3 mixtures. The solution synthesis yielded 170 A_{260} units (7.2% overall from 3′-*O*-benzoylthymidine, A_{260}/A_{280} = 1.44).

Results

Figure 1 shows the structure of thymidine. Expansions of the aromatic and methyl regions of the proton spectrum of undeuteriated TpTpTpT are shown in Figure 2. In the methyl region there were three peaks with intensities in the ratio of 1:2:1 at 1.865, 1.875, and 1.884 ppm, respectively, the middle peak being an exact overlap of two methyl resonances. These signals will be designated a, (b + c), and d. Each signal appeared as a doublet in the resolution-enhanced spectrum (J = 1.3 Hz) because of four-bond coupling to the H6 proton on the same thymine. The H6 protons appeared as quartets centered at 7.613, 7.640, 7.654, and 7.665 ppm and will be designated e, f, g, and h. The absolute chemical shifts of the methyl and H6 signals varied slightly from one sample to another because of slight differences in concentrations of the oligomer; however, the relative chemical shifts remained constant for each sample that was examined.

The methyl signals could be correlated with the H6 protons by homonuclear decoupling. Irradiation of quartet e resulted in the collapse of the a doublet, indicating that they were located on the same thymine. Similarly, irradiation of h collapsed the d doublet, and irradiations of f and g partially collapsed the overlapped doublet for b and c. However, no information could be derived from these decoupling studies or from attempted studies of nuclear Overhauser effects to correlate any of these resonances with the location of individual thymines in the sequence.

In Figure 3 are shown similar expansions of the aromatic and methyl regions of the deuteriated tetramer TpTpTpT, which had the following mole fraction of CH_3 remaining in each thymine: T_1 (5′-end), 0.25; T_2, 0.50; T_3, 0.75; T_4 (3′-end), 1.00. Integration of methyl resonances a, (b + c), and d showed them to be in the ratio of 1:1.23:0.31.[8] Substitution of three deuterium atoms into the methyl group resulted in collapse of the H6 quartet to a singlet. Resonance h centered at 7.665 ppm (coupled to the d methyl peak at 1.884 ppm) remained a full quartet, indicating that the thymine from which that resonance and the d methyl signal were derived was undeuteriated and must therefore be T_4. The other three H6 resonances were quartets of variably reduced intensities, overlain by singlets of different sizes, with the singlets displaced 0.0016 ppm upfield from the center of the quartets. Resonance e at 7.613 ppm (coupled to the a methyl at 1.865 ppm) appeared to have the least intense quartet and most intense singlet, indicating that e and a were in T_1, the thymine containing the smallest mole fraction of CH_3, (0.25 CH_3). Resonance f at 7.640 ppm was a composite of the largest quartet and smallest singlet and was accordingly assigned as T_3 (0.75 CH_3). The remaining resonance, g at 7.654 ppm, was made up of a quartet and a singlet of intermediate sizes and was assigned as T_2 (0.50 CH_3). The methyl groups for T_2 and T_3 both apppear at 1.875 ppm. It is noteworthy that because of this overlap of methyl resonances b and c assignments of H6 resonances could not be made by the alternate route of integration of the methyl signals followed by identification of allylic relationship of the methyl and H6 protons by homonuclear decoupling.

In order to test how small a difference in deuterium content of the thymine methyl groups could be reliably measured, a second partially deuteriated sample of TpTpTpT was prepared having the following mole fraction composition: T_1, 0.40; T_2, 0.53; T_3, 0.66; T_4, 1.00. The observed ratio of the methyl signals was 1:1.17:0.46, and the calculated ratio for the assignment T_4, (T_2 and T_3), T_1 was 1:1.19:0.40. Again, the assignment was supported by the spectrum of the aromatic region. The resonances were similar in appearance to those in Figure 3, but the intensities of the singlets and quartets were different. Resonance h was still a pure quartet (T_4), and f had the smallest singlet and largest quartet (T_3). Resonances e and g were similar to f, reflecting the small difference in CH_3/CD_3 content among the three, but e appeared to contain the larger singlet, consistent with its assignment as being from T_1.

Discussion

In order to assign the ^1H NMR resonances of identical bases in an oligodeoxynucleotide, the resonances must be made distinguishable in some fashion without significant structural changes, which could influence the conformation and thus change the chemical shifts of the protons in question. Isotopic substitution is a method that fulfills this criterion and is also relatively easy and inexpensive to do by catalytic exchange and chemical synthesis. Previously, the main route to deuteriated DNA and oligonucleotides has been by biological incorporation.[9]

The deuteriated nucleoside was synthesized by platinum-catalyzed exchange procedures.[5,10] It was found that the number of sites and percentage of exchange increased most dramatically with increasing ratio of catalyst to nucleoside. In the case of thymidine, Kinoshita et al.[5] achieved the most complete exchange at a temperature of 50 °C and a time of 45 h by the use of a platinum to nucleoside ratio of 10:1 on a 5-mg sample of thymidine, with excess deuterium gas. However, partial deuteriation of the C6 and the deoxyribose ring also occurred. Maeda and Kawazoe[10] developed a more selective method, with incorporation only at the methyl group, by using only a trace of deuterium gas at a temperature of 30 °C for 40 h and a platinum to nucleoside ratio of 1:2.42 on a 242-mg sample of thymidine. Kinoshita et al.[5] reported HPLC yields in the range of 70–90%; Maeda and Kawazoe[10] did not report yields.

On a preparative scale, it is desirable to limit the amount of platinum without reducing the efficiency of the reaction. We found that this goal can be achieved with only a minor sacrifice of time and yield. In the reaction described here, the initial platinum/nucleoside ratio was 0.5 g of PtO_2 to 6 g of thymidine (1:12), followed by 0.3 g of PtO_2 after 5 days. The reaction time was extended to 8 days to approach complete exchange. Maeda

(8) The number of possible different results is 4!/2 = 12; the calculated ratio for each was compared to the observed results. The closest calculated ratio was 1:1.25:0.25, for the assignment T_4, (T_3 + T_2), T_1.

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and Kawazoe[10] used only a catalytic amount of deuterium gas in a sealed tube to get 100% incorporation. Our reaction proceeded very slowly in the absence of substantial amounts of hydrogen, presumably due to the escape of the gas through the rubber septum during heating. It is significant to note that deuterium gas is not necessary for the incorporation if D_2O is used as the solvent. Hydrogen gas is dissociated and quickly exchanged with the solvent at the surface of the catalyst. In our case, a mixture of hydrogen gas (175 mL, ~0.0078 mol) and D_2O (67.8 mL, 3.39 mol) represented a >400-fold excess of D over H.[11]

In the present study, two unidentified byproducts were found in the mother liquors from the preparation of [*methyl*-$^2H_3$]thymidine. Neither appeared to be a product of the reduction of the C5–C6 thymine double bond, on the basis of a comparison of the NMR spectrum with the reported spectrum of 5,6-dihydrothymidine.[12] Reduction was not reported by either Kinoshita et al.[5] or Maeda and Kawazoe,[10] although uridine, which is less hindered, is extensively reduced under the same conditions. The crystallized yield of our preparation was 75%, with 94.2% incorporation of deuterium. This result compares favorably with previously reported procedures for the deuteriation of nucleosides, which had been done on a much smaller scale.[5,10,11]

In the partial deuteriation method described in this report, the reliability of integration is crucial. The isotopic purity of the deuteriated mononucleotide and the percentages of CD_3 annd CH_3 in each mononucleotide mixture must be precisely established during synthesis. The H to D ratio in the starting nucleoside and the mononucleotide mixtures is easily checked by integration of the appropriate peaks. For accurate integration it is essential that the individual CH_3 signals be well resolved in the spectrum. To some extent, this can be accomplished by adjustment of the temperature at which the spectrum is acquired. The spectrum of the tetramer described in this paper is an example of an unfortunate but not unlikely situation where two of the methyl signals are isochronous and the other two lie very close to them. The four methyl signals are spread over only 0.03 ppm, i.e., 12 Hz at 400 MHz. The situation is made worse by the fact that each methyl group is coupled to H6, creating a doublet of ~1.3-Hz separation. The H6 protons are also spread over only a small region, ~0.06 ppm, and the overlap problem is compounded by the fact that the H6 signals for undeuteriated species appear as quartets; however, the four of them can be resolved almost completely from one another by application of sufficient Gaussian resolution enhancement. Although it may not be possible to completely resolve the spectrum, as is the case for TpTpTpT, a knowledge of the exact isotopic ratio at each nucleotide can be compared with calculated spectra to arrive at the proper assignment.[13]

A possible source of error arises from incomplete incorporation of deuterium into the thymine mononucleotide. We utilized an analysis of isotope shifts to examine whether the residual protons in the deuteriated thymidine were present entirely as CH_3 or randomly distributed as CH_3, CH_2D, and CHD_2. The methyl carbon signal in the ^{1}H-decoupled ^{13}C NMR spectrum of [*methyl*-$^2H_3$]thymidine, which is 70% deuteriated, is a complex overlapping pattern in which 14 of the 16 peaks arising from the four possible species are visible (CH_3 = 1 peak, CH_2D = 3, CHD_2 = 5, and CD_3 = 7; $J_{C,D}$ = 19.8 Hz). The addition of each succeeding deuterium increases the upfield isotope shift approximately 5 Hz from CH_3 so that each multiplet is distinct from the others. Only the outermost lines of the CD_3 heptet are not clearly visible. From analysis of the relative areas of the multiplets we conclude that the residual protons are randomly distributed.[14]

In conclusion, we have demonstrated the usefulness of partial deuteriation as a method for identifying the different thymine resonances in the ^{1}H NMR spectrum of the tetradeoxynucleotide TpTpTpT.[15] Two experiments were done in order to determine the smallest difference between the percentages of CH_3 that could reliably be detected. From our results, it seems likely that as many as seven thymine methyls could be identified in a single NMR experiment, if the ratios used were 100:85:70:55:40:25:10, although overlaps, as were encountered in the case of TpTpTpT, could limit the number of thymines that could be distinguished.[18] The likelihood of overlaps is greatest in homooligomers; however, where other bases are present, the magnetic environment of each methyl group is more likely to be different.

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Registry No. d(TpTpTpT), 2476-57-5; 2′-deoxy-5-(trideuteriomethyl)uridine, 74848-84-3; thymidine, 50-89-5.

(11) Lawson, J. A.; DeGraw, J. I. In *Nucleic Acid Chemistry*, Part 2; Townsend, L. B., Tipson, R. S., Eds.; Wiley: New York, 1978; pp 921–926. Lawson and DeGraw, who prepared [6-^{13}C-α,α,α-$^2H_3$-1,3-$^{15}N_2$]thymidine from the appropriately labeled (cyanopropionyl)urea, tried to incorporate deuterium at the C6 position in the reductive cyclization step by using deuterium gas in H_2O. No incorporation of deuterium was detected. Our experience suggests that this reaction should succeed in D_2O.

(12) Hanze, A. R. *J. Am. Chem. Soc.* **1967**, *89*, 6720.

(13) Minimum Gaussian parameters were chosen to give clean separation without causing the skirts of peaks to dip below the base line or for significant changes to occur in relative peak areas. Apodization functions will alter the relative peak areas in cases where the signals being compared have different spin–spin relaxation time constants. However, in the present case the relative areas for the methyl groups were not sensitive to Gaussian parameters, indicating similar relaxation times.

(14) A similar, although less well-defined pattern, is observed for the spectrum of 94% methyl-deuteriated thymidine, in which the heptet is the main contributor. The ^{1}H spectrum confirms this observation. The residual methyl protons are found in a small triangle-shaped peak, skewed upfield, with its apex at 1.854 ppm. The direction of the skew indicates that the main proton-bearing species is CD_2H, followed by CDH_2 and CH_3. The doublet in nondeuteriated thymidine is centered at 1.884 ppm. Therefore, the residual protons in the three deuteriated methyl groups in TpTpTpT are displaced 0.03 ppm upfield, beyond the methyl signals in question; hence, the contribution of residual protons to the integral of the methyl peaks can be safely discounted.

(15) The method is applicable to deoxyadenosine and deoxycytidine, as well as thymidine, and should be equally useful with ribonucleosides. The only requirement is that the deuterium, once introduced, not be removed by any step in the oligonucleotide synthesis. In addition to deuterium substitution of the methyl group of thymidine described herein, we have been able to substitute deuterium at the C2 position of deoxyadenosine to the extent of 72% on a preparative scale. Deuteriation of the C8 position of deoxyguanosine and deoxyadenosine is of no value for the preparation of oligodeoxynucleotides, since deuterium exchanged into the C8 position of purine nucleosides is lost in the ammonia deblocking step in the synthesis of oligomers. Deoxycytidine is a special case. We have prepared 5-deuteriodeoxycytidine (C5 D = 95%, C6 D = 13%) by Pt-catalyzed exchange in D_2O at pH 3.5 and 5,6-dideuteriodeoxycytidine (C5 D = 95%, C6 D = 83%) by exchange in D_2O at pH 7.0. Treatment of the dideuterio material with NH_4HSO_3[16] will exchange the C5 position back to H, thereby affording 6-deuteriodeoxycytidine. Thus by careful control of exchange conditions, 5-deuterio-, 6-deuterio-, or 5,6-dideuteriodeoxycytidine may be obtained. Exchange reactions for the C6 positions of pyrimidines have been reported that utilize strong bases.[17]

(16) Kai, K.; Wataya, Y.; Hayatsu, H. *J. Am. Chem. Soc.* **1971**, *93*, 2089.

(17) (a) Rabi, J. A.; Fox, J. J. *J. Am. Chem. Soc.* **1973**, *95*, 1628. (b) Pichat, L.; Godbillon, J.; Herbert, M. *Bull. Chim. Soc. Fr.* **1973**, 2715.

(18) A recent paper proposes the formation of a parallel, right-handed duplex of TpTpTpTpTpT containing phosphotriester linkages: Koole, L. H.; van Genderen, M. H. P.; Buck, H. M. *J. Am. Chem. Soc.* **1987**, *109*, 3916. The authors were not able to assign any of the thymidine methyl resonances in the ^{1}H NMR spectrum, which were found in three peaks in the ratio 1:1:4. Their arguments for the structure were based in part upon the assumption that the individual signals correspond to the 3′- and 5′-terminal thymidine units. Inclusion of partially deuteriated thymidine at the 3′- and 5′-terminals would provide a direct method of assigning the various thymine resonances.