Raman and Infrared Spectra of (2′*S*)-[2′-^{2}H]Thymidine: Vibrational Coupling between Deoxyribosyl and Thymine Moieties and Structural Implications

Masamichi Tsuboi,*,† Mamoru Komatsu,‡ Junichi Hoshi,‡ Etsuko Kawashima,§ Takeshi Sekine,§ Yoshiharu Ishido,§ Malcolm P. Russell,⊥ James M. Benevides,⊥ and George J. Thomas, Jr.*,⊥

Contribution from the Department of Fundamental Science, Iwaki−Meisei University, Iwaki, Fukushima 970, Japan, Nicolet Japan Corporation, Hatsudai 2-5-8, Shibuya-ku, Tokyo 151, Japan, Laboratory of Pharmaceutical Chemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan, and School of Biological Sciences, University of Missouri, Kansas City, Missouri 64110

Received August 1, 1996[⊗]

Abstract: The thymidine stereoisotopomer, (2′*S*)-[2′-^{2}H]thymidine, which incorporates deuterium in the *S* configuration at the furanosyl 2′ carbon, has been synthesized and its vibrational spectra have been recorded and compared with those of normal thymidine. Infrared and Raman spectra were collected from crystalline powders, the latter using 1063- and 514.5-nm excitations; ultraviolet resonance-Raman spectra were collected from aqueous solutions using 244-nm excitation. The results show, remarkably, that virtually all normal modes of thymidine involve some degree of vibrational coupling between the thymine base residue and the deoxyribose moiety. Nevertheless, systematic assignments and correlation of the spectral frequencies of thymidine and (2′*S*)-[2′-^{2}H]thymidine have been accomplished. A finding of importance for nucleic acid structure applications is that many prominent Raman marker bands of thymidine, assigned previously as thymine ring vibrations, in fact involve appreciable coupling with the C2′ methylene group of the attached sugar. Vibrational coupling between the base and sugar groups implies frequency dependence upon sugar conformation and allows the bands in question to be exploited as markers of deoxyribose ring pucker and glycosyl orientation in Raman spectra of DNA, antiviral drugs, and other thymine-containing nucleoside analogues. The present results also enable unambiguous and novel assignment of spectral bands to specific vibrational modes of the C2′ methylene group of thymidine as follows: C2′H_2 antisymmetric stretching (2995 cm^{-1}), symmetric stretching (2956 cm^{-1}), scissoring (1404 cm^{-1}), and wagging (1174 cm^{-1}). Additionally, probable assignments are deduced for the C2′H_2 twisting (1103 cm^{-1}) and rocking modes (898 cm^{-1}). Normal mode assignments are also proposed for many other vibrational bands of thymidine.

Introduction

Thymidine, as one of the four nucleoside constituents of DNA, is of fundamental importance in molecular biology. Repeats of thymidine, which occur in the specialized sequences of telomeric DNA, fulfill additional functions in the supramolecular organization of linear eukaryotic chromosomes.[1] Thymidine is also an essential constituent of many pharmacological agents, including the antiviral compounds azidothymidine (AZT) and dideoxythymidine (ddT).[2] Vibrational spectroscopy serves as a convenient and effective probe of thymidine structure and interaction in these biological molecules, and the thymine base itself is an intriguing target for vibrational analysis. Raman studies have demonstrated a rather unique pattern of thymine vibrational bands in the spectral interval 640−800 cm^{-1}, clearly differentiating thymine nucleosides and nucleotides from those of uracil and cytosine.[3−5]

Thymidine is alone among pyrimidine nucleosides in exhibiting multiple Raman markers of prominent intensity in the 640−800-cm^{-1} interval. These thymidine vibrational bands are apparently sensitive to the conformation and structural context of the nucleoside, as has been demonstrated in thymidine-containing mononucleotides[5] and nucleic acids.[6−8] Conversely, uridine and cytidine derivatives generate a single Raman marker near 780 cm^{-1}, which is largely insensitive to changes in conformation or environment of the nucleoside moiety.[9−11]

In order to exploit vibrational spectroscopy as a structural probe of thymidine-containing nucleic acids, it is of interest to determine whether the conformational dependence of thymidine Raman marker bands originates from vibrational coupling between the base and sugar moieties or is reflective of interactions involving base exocyclic groups. For this purpose

* Authors to whom correspondence may be addressed.
† Iwaki−Meisei University.
‡ Nicolet Japan Corporation.
§ Tokyo University of Pharmacy and Life Science.
⊥ University of Missouri.
⊗ Abstract published in *Advance ACS Abstracts,* February 1, 1997.

(1) Henderson, E. *Telomeres*; Blackburn, E. H., Greider, C. W., Eds.; CSHL Press: Cold Spring Harbor, NY, 1995; pp 11−34.
(2) Martin, J. C., Ed. *Nucleotide Analogues as Antiviral Agents*; ACS Symp. Ser., Vol. 401; American Chemical Society: Washington, DC, 1989.
(3) Hartman, K. A.; Lord, R. C.; Thomas, G. J., Jr. *Physico-Chemical Properties of Nucleic Acids*, Duchesne, J., Ed.; Academic Press: New York, 1973; Vol. 2; pp 1−89.
(4) Erfurth, S. C.; Peticolas, W. L. *Biopolymers* **1975**, *14*, 247−264.
(5) Dijkstra, S.; Benevides, J. M.; Thomas, G. J., Jr. *J. Mol. Struct.* **1991**, *242*, 283−301.
(6) Thomas, G. J., Jr.; Benevides, J. M. *Biopolymers* **1985**, *24*, 1101−1105.
(7) Katahira, M.; Nishimura, Y.; Tsuboi, M.; Sato, T.; Mitsui, Y.; Iitaka, Y. *Biochim. Biophys. Acta* **1985**, 256−267.
(8) Miura, T.; Thomas, G. J., Jr. *Biochemistry* **1995**, *34*, 9645−9653.
(9) Erfurth, S. C.; Kiser, E. J.; Peticolas, W. L. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 938−941.
(10) Nishimura, Y.; Tsuboi, M.; Sato, T.; Aoki, K. *J. Mol. Struct.* **1986**, *146*, 123−153.
(11) Prescott, B.; Steinmetz, W.; Thomas, G. J., Jr. *Biopolymers* **1984**, *23*, 235−256.

S0002-7863(96)02676-5 CCC: $14.00

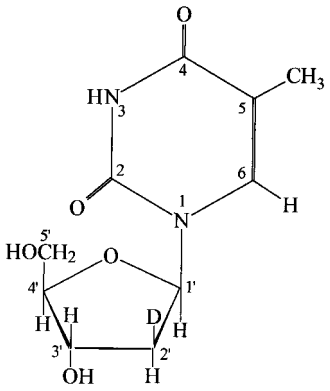

Figure 1. Covalent structure of (2′*S*)-[2′-^{2}H]thymidine [or thymidine-(2′*S*)-*d*], which incorporates deuterium (D) in the *S* configuration at the furanosyl 2′ carbon.

we have synthesized and examined the vibrational spectra of deuterium isotopomers of thymidine, with the expectation that vibrational coupling if present would be perturbed or eliminated by the isotope substitutions. In the present paper, we report the results obtained on the thymidine stereoisotopomer, (2′*S*)-[2′-^{2}H]thymidine, which incorporates deuterium (^{2}H or D) in the *S* configuration at the furanosyl 2′ methylene carbon. This structure is depicted in Figure 1.

The strategy of site-specific furanose deuteration was exploited recently by Harada and co-workers,[12] who investigated the coupling of base and sugar vibrations in purine ribonucleosides. These authors synthesized and obtained vibrational spectra of adenosine and guanosine incorporating deuterium at the C1′ carbon, which is the site of attachment of the purine base. Our results, obtained for a stereospecifically labeled C2′ deoxynucleoside, are discussed in relation to the model for base−ribose vibrational coupling proposed by Toyama et al.[12]

An additional objective of the present study is to improve our understanding of the vibrational modes of the C2′ methylene group in deoxyribonucleosides and DNA. Recent polarized Raman studies of DNA single crystals and oriented fibers show that measurably different vibrational frequencies and Raman tensors apply to the C2′ and C5′ methylene groups of the DNA backbone.[13,14] However, the previous studies do not establish which of the observed bands should be assigned to the respective methylene groups. The direct deuteration of the C2′ furanosyl site accomplished in the present work enables direct and unambiguous assignment of the characteristic vibrational modes of the C2′ methylene group. By default, the corresponding assignments are inferred for the C5′ methylene group.

Materials and Methods

Normal thymidine (D-thymidine) was purchased from Sigma Chemical (St. Louis, MO) and used without further purification.

(2′*S*)-[2′-^{2}H]Thymidine (Figure 1) was synthesized using the following six-step procedure: (1) 1-*β*-(3,5-*O*-TPDS-D-arabinofuranosyl)-thymine was oxidized with a 1:1:2 complex of CrO_3:Ac_2O:pyridine. (2) The resulting 2′-oxo derivative was deuterio-reduced stereoselectively to the corresponding 1-*β*-(3,5-*O*-TPDS-D-[2-^{2}H]arabinofuranosyl)thymine using NaB^2H_4, then (3) treated with trifluoromethanesulfonyl chloride to yield 1-*β*-(2-*O*-Tf-3,5-*O*-TPDS-D-[2′-^{2}H]arabinofuranosyl)thymine, and subsequently (4) brominated with LiBr to give (2′*R*)-2′-bromo-3,5-*O*-TPDS-[2′-^{2}H]thymidine. (5) Highly stereoselective reduction of the last intermediate with the $Bu_3SnH-Et_3B$ system at low temperature, and finally (6) unmasking, yielded the desired product. Further details of this synthesis are given elsewhere.[15] Isotopic purity of the product was assessed as 98.3% ^{2}H by examination of the 400-MHz NMR spectrum, which also indicated >95% *S* configuration at the 2′ position.

Infrared and Raman (1063 nm excitation) spectra of thymidine and (2′*S*)-[2′-^{2}H]thymidine were obtained from crystalline powders on a Nicolet FTIR/FT-Raman spectrometer. Additional Raman spectra (514.5-nm excitation) of the same crystalline powders were obtained on a Jobin-Yvon ISA S3000 spectrometer equipped with an Olympus microscope. Ultraviolet resonance Raman (UVRR) spectra were obtained from aqueous solutions (0.5 mg/mL) in a rotating cell using 244-nm excitation. Further details of the Raman microscope and UVRR spectrometer assemblies have been given elsewhere.[13,16]

Results

Infrared and Raman spectra (1063-nm excitation) of thymidine and (2′*S*)-[2′-^{2}H]thymidine [hereafter referred to as thymidine-(2′*S*)-*d*] in the regions 1800−1300 and 1300−650 cm^{-1} are compared in Figures 2 and 3, respectively. Corresponding spectra were also obtained for the hydrogen stretching region (2900−3020 cm^{-1}) and deuterium stretching region (2100−2300 cm^{-1}) and are available as Supporting Information or by request from the authors. Key bands assigned to C2′H and C2′D stretching modes are included in Table 1. Raman spectra for the region 100−650 cm^{-1} are presented in Figure 4. (Infrared spectra below 650 cm^{-1} were not obtained.) Comprehensive Raman spectra (200−3100-cm^{-1}, 514.5-nm excitation) of thymidine and thymidine-(2′*S*)-*d*, and their digitally computed difference spectrum, are presented in Figure 5. The very low frequency region of the Raman spectrum (down to 50 cm^{-1}) was also recorded and is available as Supporting Information or upon request from the authors.

UVRR spectra (244-nm excitation) of thymidine and thymidine-(2′*S*)-*d*, and their computed difference spectrum, are given in Figure 6.

Discussion

1. General Survey of the Vibrational Spectra. The thymidine molecule has 31 atoms and 87 normal modes of vibration, including 14 hydrogen stretching vibrations expected to have frequencies above 2800 cm^{-1}. The remaining 73 vibrations are expected with frequencies below 1800 cm^{-1}. The thymidine crystal (space group $P2_12_12_1$)[17] is subject to different selection rules for infrared absorption and Raman scattering. Accordingly, somewhat different vibrational frequencies may occur in the infrared and Raman spectra of the crystalline powder. A survey of Figures 2 and 3 confirms that this is indeed the case for a few of the vibrational modes. For example, we find no Raman counterpart to the strong infrared band of thymidine-(2′*S*)-*d* at 1078 cm^{-1}. However, most of the prominent infrared bands have counterparts of nearly the same frequencies in the Raman spectrum, and *vice versa*. For both thymidine and thymidine-(2′*S*)-*d*, 60 non-redundant vibrational frequencies are observed in the 70−1800 cm^{-1} region. The very lowest frequency region of the crystal spectrum (Supporting Information) reveals additional bands probably due to lattice librational modes.

The data of Figures 2−6 demonstrate great sensitivity of the vibrational signature of thymidine to C2′ deuteration. This is especially evident in the difference spectrum shown in Figure

(12) Toyama, A.; Takino, Y.; Takeuchi, H.; Harada, I. *J. Am. Chem. Soc.* **1993**, *115*, 11092−11098.
(13) Benevides, J. M.; Tsuboi, M.; Wang, A. H.-J.; Thomas, G. J., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 5351−5359.
(14) Thomas, G. J., Jr.; Benevides, J. M.; Overman, S. A.; Ueda, T.; Ushizawa, K.; Saitoh, M.; Tsuboi, M. *Biophys. J.* **1995**, *68*, 1073−1088.
(15) Kawashima, E.; Aoyama, Y.; Miyahara, M.; Sekine, T.; Radwan, M.; Ishido, Y. *Nucleosides Nucleotides* **1995**, *14*, 333−336.
(16) Russell, M.; Vohník, S.; Thomas, G. J., Jr. *Biophys. J.* **1995**, *68*, 1607−1612.
(17) Young, D. W.; Tollin, P.; Wilson, H. R. *Acta Crystallogr.* **1969**, *B25*, 1423−1432.

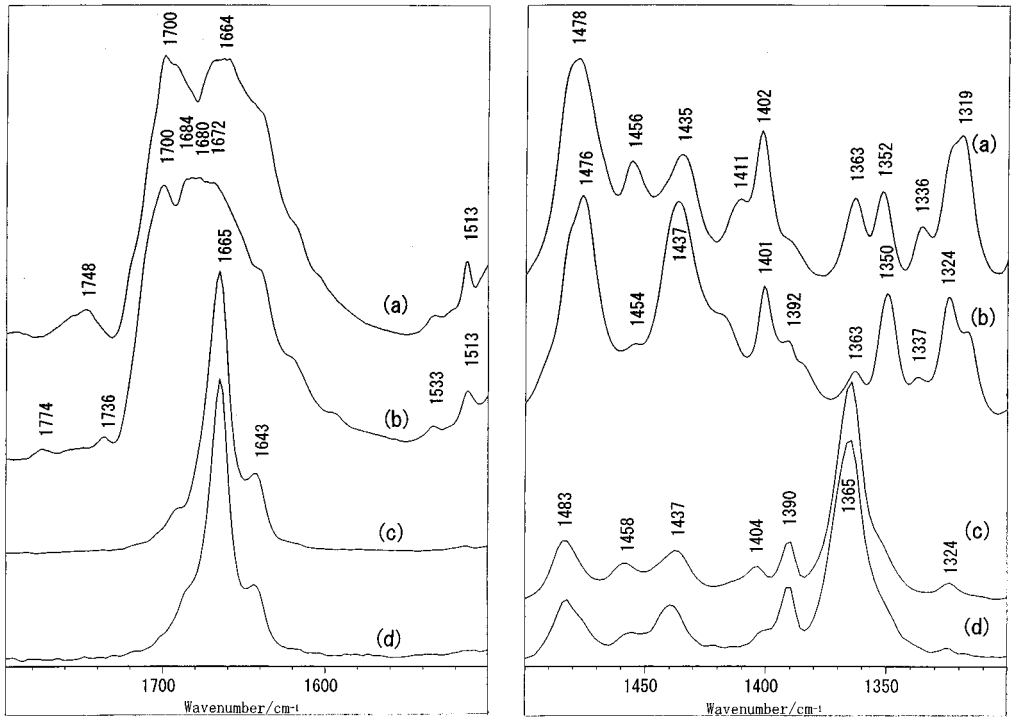

Figure 2. Infrared spectra of crystalline powders of thymidine (trace a) and thymidine-(2′*S*)-*d* (trace b) and Raman spectra (1063-nm excitation) of crystalline powders of thymidine (trace c) and thymidine-(2′*S*)-*d* (trace d). Left panel: Region 1800−1500 cm^{-1}. Right panel: Region 1500−1300 cm^{-1}.

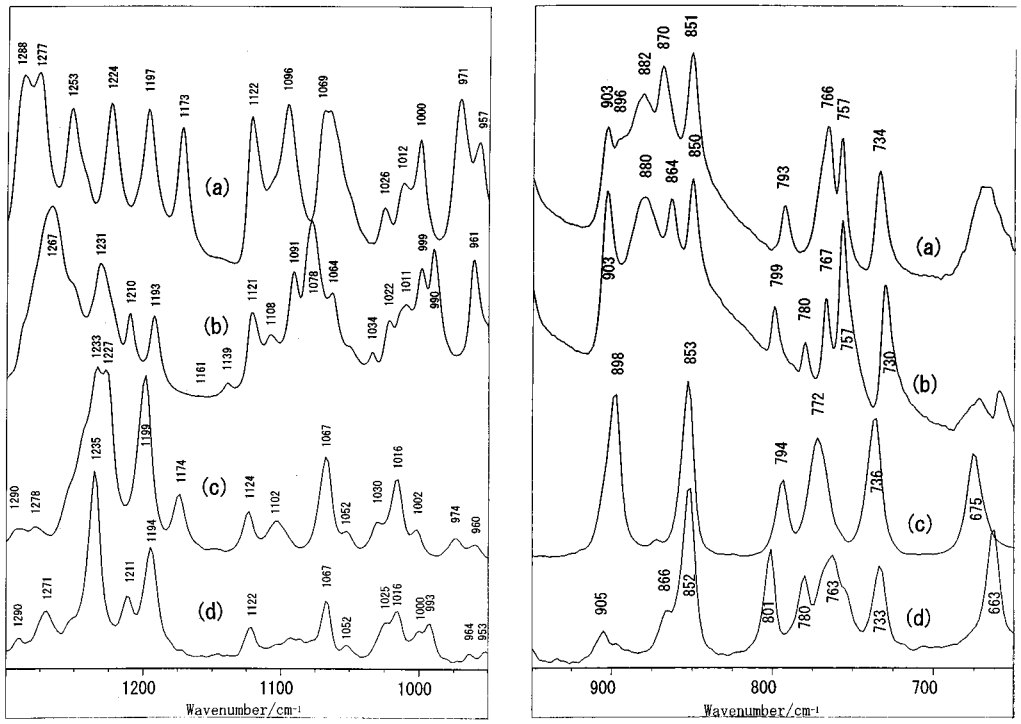

Figure 3. Infrared spectra of thymidine (trace a) and thymidine-(2′*S*)-*d* (trace b) and Raman spectra of thymidine (trace c) and thymidine-(2′*S*)-*d* (trace d). Other conditions are as given in Figure 2. Left panel: Region 1300−950 cm^{-1}. Right panel: Region 950−650 cm^{-1}.

5. Remarkably, of the 70 or so vibrational bands observed in the interval 100−3000 cm^{-1}, about 50 are clearly perturbed by C2′ deuteration. Shifts to lower frequency as large as 12 cm^{-1} are observed. The molecular vibrations giving rise to the shifted bands involve some contribution from the C2′-D group. Some bands, however, appear unique to either normal thymidine or thymidine-(2′*S*)-*d*, and are thus assignable respectively to localized vibrations of either the C2′H$_2$ group of thymidine or the C2′HD group of thymidine-(2′*S*)-*d*. These bands are listed in Table 1 along with their proposed vibrational assignments.

In the UVRR spectrum (244-nm excitation, Figure 6) of thymidine only resonance-enhanced Raman bands of the thymine ring are expected. The UVRR frequencies observed for aqueous thymidine and thymidine-(2′*S*)-*d* are listed in the first two columns of Table 2. Also included in Table 2 are the frequencies of bands that are observed in off-resonance Raman spectra (1063 nm) and infrared absorption spectra of the crystalline powders and assigned to vibrations largely within the thymine moiety. As seen here, the six prominent bands in the interval 1190−1700 cm^{-1} exhibit little or no deuteration

Table 1. Vibrational Bands of the C2′-Methylene Group of Thymidine

bands of the C2′H_2 species			bands of the C2′H(*S*)D species		
infrared[a]	Raman[a]	assignment[b]	infrared[a]	Raman[a]	assignment[b]
2994 (m)	2996 (s)	CH_2 antisym st	2984 (w)	2984 (s)	C−H st
2956 (w)	2956 (m)	CH_2 sym st	2187 (w)	2187 (m)	C−D st
1410 (w)	1404 (w)	CH_2 scissor	1078 (s)		C2′DH def
1173 (s)	1174 (m)	CH_2 wag	990 (s)	993 (m)	C2′DH def
1069 (s)	1103 (w)	CH_2 twist	903 (m)	905 (m)	C2′DH def
896 (w)	898 (s)	CH_2 rock	799 (m)	801 (m)	C2′DH def

[a] Band frequencies are given in wavenumber units (cm^{-1}) and relative intensities are indicated in parentheses. Abbreviations: s strong, m medium, w weak. [b] Detailed assignments and further discussion are given in the text. Abbreviations antisym antisymmetric, st stretch, def deformation.

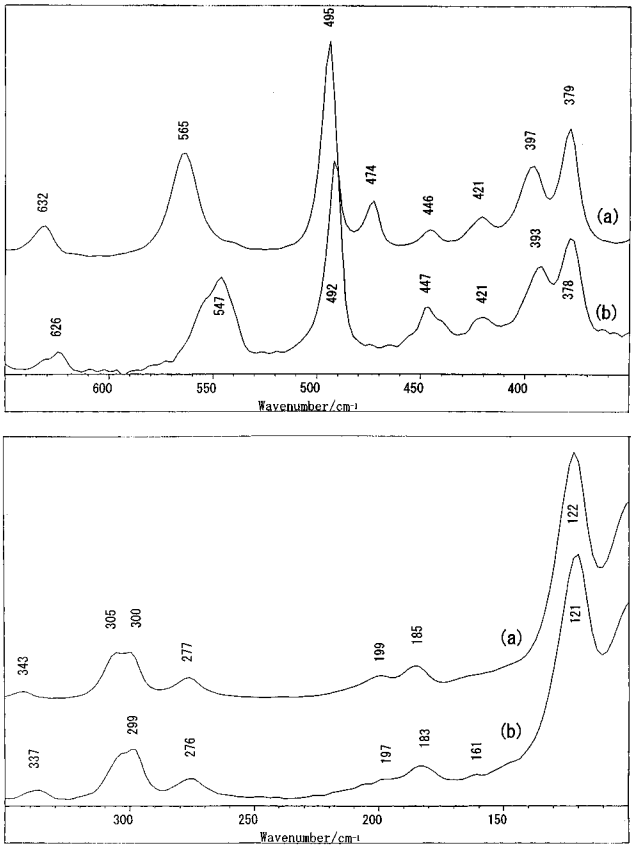

Figure 4. Raman spectra (1063-nm excitation) of crystalline powders of thymidine (trace a) and thymidine-(2′*S*)-*d* (trace b) in the region 650−100 cm^{-1}.

shifts, indicating no significant involvement of C2′H_2 (or C2′HD) motions. However, the UVRR bands of lower frequency (600−1190 cm^{-1}) show appreciable deuteration shifts. Therefore, the latter are assignable to more highly delocalized vibrations, extending into the deoxyribose moiety and exhibiting sensitivity to C2′ deuteration. The present Raman data are complemented in Table 2 by recently published Raman spectra (488.0 nm) obtained on thymidine-$^{13}C_5$, in which the furanose carbons have been isotopically substituted with ^{13}C.[18] The deuterium and ^{13}C shifts are generally consistent and indicative of the extent of vibrational coupling between thymine and deoxyribose moieties.

On the basis of the foregoing results, detailed vibrational characterizations can be proposed for many of the observed Raman and infrared bands of thymidine, as next discussed.

(18) Tsuboi, M.; Ueda, T.; Ushizawa, K.; Sasatake, Y.; Ono, A.; Kainosho, M.; Ishido, Y. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1483−1484.

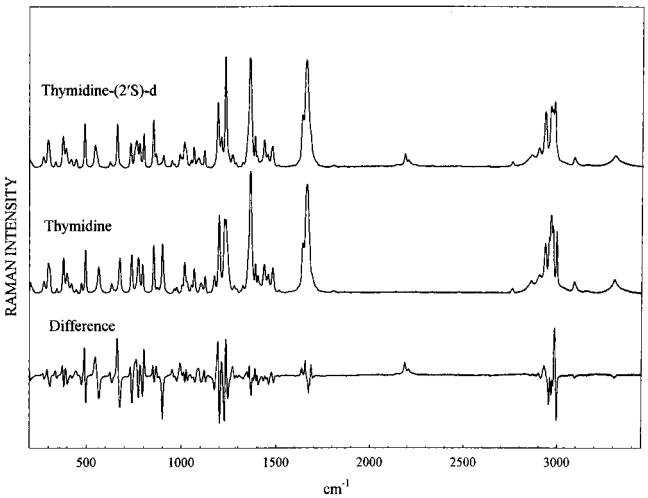

Figure 5. Raman spectra (514.5-nm excitation) of crystalline powders of thymidine-(2′*S*)-*d* (top) and thymidine (middle) in the region 200−3500 cm^{-1} and their digitally computed difference spectrum (bottom). (A complete tabulation of the spectral frequencies is available from the authors upon request.)

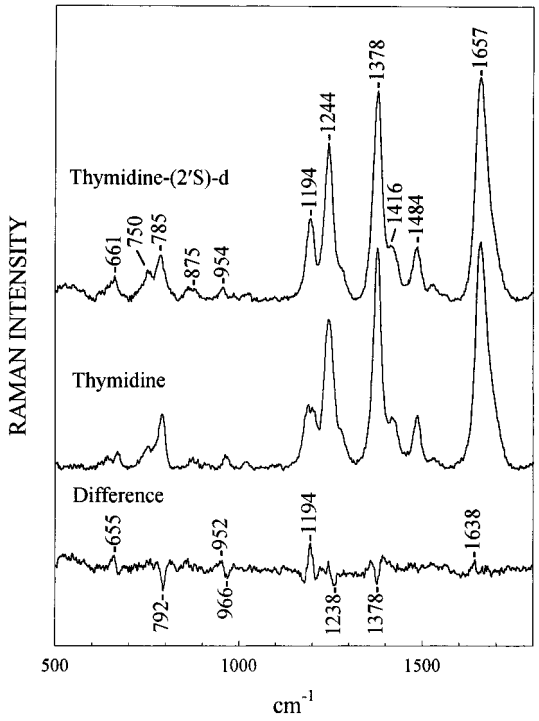

Figure 6. UV-resonance Raman spectra (244-nm excitation) of aqueous solutions of thymidine-(2′*S*)-*d* (top) and thymidine (middle) in the 500−1800-cm^{-1} region and their computed difference spectrum (bottom). Nucleoside concentration is 0.5 mg/mL (pH 7).

2. Vibrational Assignments for the C2′H_2 Group. a. Stretching Vibrations. Thymidine bands at 2956 and near 2995 cm^{-1} disappear upon 2′(*S*) deuteration and are assigned respectively to symmetric and antisymmetric C−H stretching vibrations of the C2′H_2 group (Table 1). The replacement bands of thymidine-(2′*S*)-*d* at 2984 and 2187 cm^{-1} are assigned respectively to the C2′(*R*)−H and C2′(*S*)−D stretching modes of the C2′HD group (Table 1). Each stretching vibration exhibits essentailly the same frequency in Raman and infrared spectra.

b. Scissoring Vibration. The weak Raman band of thymidine at 1404 cm^{-1} (Figure 2) has been assigned to CH_2 scissoring on the basis of its ^{13}C isotope shift of −5 cm^{-1}.[18] The intensity loss accompanying C2′(*S*) deuteration confirms

Table 2. Vibrational Frequencies of the Thymine Base in Thymidine and Thymidine-(2′*S*)-*d*

UVRR spectra (244 nm)[a]			Raman spectra (1063 nm)[b]				infrared spectra[c]		
C2′H_2	C2′(*S*)D	$\delta(^2H)$[d]	C2′H_2	C2′(*S*)D	$\delta(^2H)$[d]	$\delta(^{13}C)$[e]	C2′H_2	C2′(*S*)D	$\delta(^2H)$[d]
1657(s)	1657(s)	0	1665(s)	1665(s)	0	0			
1486(m)	1484(m)	−2	1483(m)	1483(m)	0	−1	1478(s)	1476(s)	−2
1416(w)	1416(w)	0	1390(m)	1390(m)	0	0	1402(m)	1401(m)	−1
1377(s)	1378(s)	+1	1365(s)	1365(s)	0	−1	1363(w)	1363(w)	0
1244(s)	1244(s)	0	1227(s)	1235(s)	+2	−2			
			1233(s)						
1198(m) } 1188(m) }	1194(m)	−2	1199(s)	1194(s)	−5	−3	1197(s)	1193(m)	−4
961(w)	954(w)	−7	960(w)	953(w)	−7		971(s)	961(s)	−10
							870(m)	864(m)	−6
875(w)	875(w)	0	853(s)	852(s)	−1	−12	851(m)	850(s)	−2
789(m)	785(m)	−4	772(s)	763(s)	−9	−1	766(s)	767(m)	+1
							757(s)	757(s)	0
752(w)	750(w)	−2	736(s)	733(s)	−3	−10	734(s)	730(s)	−4
668(w)	661(w)	−7	675(s)	663(s)	−12	−4	666(s)	664(w)	−2

[a] Data from Figure 6. Other notation as in Table 1. [b] Data from Figures 2 and 3 and ref 18. Other notation as in Table 1. [c] Data from Figures 2 and 3. Other notation as in Table 1. [d] Frequency shift with C2′(*S*) deuteration. [e] Frequency shift with ^{13}C substitution of deoxyribose carbons.[18]

the earlier assignment and shows further that the band originates primarily from the C2′ methylene group. The likely infrared counterpart is the weak band at 1411 cm^{-1}, which essentially disappears with C2′(*S*) deuteration. In spectra of thymidine-(2′*S*)-*d*, a Raman band occurs near 1400 cm^{-1}. This frequency may be assigned to the C5′H_2 scissoring mode. The corresponding Raman band for the nondeuterated nucleoside is obscured by the stronger C2′H_2 scissoring bands expected in this region.

The medium intensity infrared band, which remains at approximately 1402 cm^{-1} upon C2′(*S*) deuteration, presumably corresponds to the Raman band near 1390 cm^{-1}, which also remains unchanged by C2′ (*S*) deuteration. Both are assigned to the thymine ring (Table 2) and not to methylene scissoring modes.

c. Wagging Vibration. The strong infrared band at 1173 cm^{-1} and medium-intensity Raman band at 1174 cm^{-1} in the spectra of undeuterated thymidine are both eliminated by C2′-(*S*) deuteration (Figure 3). Assignment to the CH_2 wagging vibration is supported by the fact that the 1174-cm^{-1} Raman band is shifted by −13 cm^{-1} to 1161 cm^{-1} upon ^{13}C substitution of the deoxyribose carbons.[18]

According to Nakagawa and Mizushima,[19] the diagonal element of the ***G*** matrix for the C2′H_2 wagging vibration is given by

$$\boldsymbol{G}(\text{C2′H}_2\text{ wag}) = [(3/2)\mu_H + 3\mu_C]r_{CH}^2 + (1/2)\mu_C r_{CC}^2 + \mu_C[(1/3)r_{CC}^2 + 2r_{CH}r_{CC}] \quad (1)$$

where μ_H and μ_C are reciprocals of the atomic masses of H and C, respectively, and r_{CH} and r_{CC} are bond lengths. With r_{CH} = 1.1 Å and r_{CC} = 1.5 Å, eq 1 predicts the ratio ***G***(^{12}C2′H_2 wag)/***G***(^{13}C2′H_2 wag) = 1.0227 and a corresponding vibrational frequency ratio of 1.0113, which is identical to the observed 1174/1161 cm^{-1} ratio of Raman frequencies. (Similar calculation of the ratio of ***G*** matrix elements for C2′H_2 twisting and rocking vibrations leads to predicted ^{13}C-isotope shifts of −6 and −20 cm^{-1}, respectively.) Therefore, the 1174-cm^{-1} band can be assigned confidently to the wagging vibration.

d. Twisting Vibration. This vibration is expected in the 1000−1300-cm^{-1} region. As seen in Figure 3, a weak Raman band at 1102 cm^{-1} disappears on C2′(*S*) deuteration and the strong infrared band at 1096 cm^{-1} is weakened. These effects are attributable to the C2′H_2 twisting mode. The weak infrared and Raman bands remaining near 1091 cm^{-1} may be assigned to the C5′H_2 twisting mode.

e. Rocking Vibration. The strong Raman band at 898 cm^{-1} was assigned previously to a deoxyribose ring breathing vibration on the basis of its ^{13}C-isotope shift of −17 cm^{-1}.[18] The present data show, however, that the 898-cm^{-1} band is sensitive to C2′(*S*) deuteration (Figure 3). Therefore, it is now clear that the vibration in question involves a C2′H_2 motion, probably methylene rocking. Because no band in this region of the thymidine spectrum exhibits the larger ^{13}C shift (−20 cm^{-1}) expected for a pure methylene rocking mode, it is further concluded that thymidine has no pure methylene rocking mode. The C2′H_2 rocking mode is likely coupled with deoxyribose skeletal modes, and may contribute to several bands in the 700−1000 cm^{-1} region. It is suggested, however, that the C2′H_2 contribution is especially large for the 898-cm^{-1} vibration.

3. Vibrational Assignments for the C2′HD Group. a. Stretching Vibrations. Assignments of the C2′−H and C2′−D stretching vibrations of thymidine-(2′*S*)-*d* are straightforward, as given in Table 1. Interestingly, both the C2′−H and C2′−D stretching modes give rise to relatively intense Raman bands, at 2984 and 2187 cm^{-1}, respectively, whereas the corresponding infrared bands are relatively weak.

b. Deformation Vibrations. In thymidine-(2′*S*)-*d*, four deformation vibrations of the C2′H(*S*)D group are expected to replace the C2′H_2 scissoring, wagging, twisting, and rocking modes of thymidine. These are in-plane and out-of-plane CH bending and in-plane and out-of-plane CD bending modes, where the plane of reference contains the atoms H, D, and C2′. The four prospective bands of the infrared spectrum are listed in Table 1. The strong infrared band at 1078 cm^{-1} is assigned to the in-plane CH bending mode. The corresponding Raman band, which is expected to be extremely weak, is not detected in present spectra (Figure 3). The medium intensity infrared band at 799 cm^{-1} may originate from coupling between C2′−D bending and a skeletal mode of the furanose ring. The corresponding band in the Raman spectrum of thymidine-(2′*S*)-*d* occurs at 801 cm^{-1}. Such coupling would explain the shift of the 793-cm^{-1} infrared (794-cm^{-1} Raman) band of thymidine to 780 cm^{-1} in thymidine-(2′*S*)-*d* (Figure 3).

4. Vibrational Assignments for the Thymine Ring. a. Ring Modes Near 1199 and 1230 cm^{-1}. Two strong Raman bands, near 1199 and 1230 cm^{-1}, have been assigned to vibrations involving stretching of the exocyclic C5−CH_3 bond

(19) Nakagawa, I.; Mizushima, S. *Bull. Chem. Soc. Jpn.* **1955**, *28*, 589−594.

and are considered characteristic of the thymine residue.[20] In the Raman spectrum of solid undeuterated thymidine (Figure 3), the higher frequency marker occurs as a closely spaced doublet (1227/1233 cm^{-1}) which merges into a singlet (1235 cm^{-1}) upon C2′(*S*) deuteration. The 1227/1233-cm^{-1} doublet may be ascribed to Fermi resonance involving the 675 + 565 cm^{-1} combination mode. Elimination of this Fermi interaction and simultaneous introduction of another (doublet at 1194/1211 cm^{-1}) in the C2′(*S*) derivative can then be explained by the lowering of the 675- and 565-cm^{-1} frequencies to 663 and 547 cm^{-1}, respectively (Figures 3 and 4). The Fermi interaction is also eliminated by ^{13}C substitution of the five furanose carbons.[18]

It is interesting to note that in the UVRR spectrum of thymidine the thymine marker expected near 1199 cm^{-1} occurs as a doublet at 1188/1198 cm^{-1} and is replaced by a singlet at 1194 cm^{-1} in thymidine-(2′*S*)-*d* (Figure 6). The 1188/1198 cm^{-1} doublet could also be due to Fermi resonance involving a combination of the 668-cm^{-1} UVRR mode with another fundamental, presumably near 525 cm^{-1} but too weak to be observed in the UVRR spectrum. However, an alternative explanation for the 1188/1198-cm^{-1} UVRR doublet is the following. In aqueous thymidine, the C2′H_2 wagging vibration and a thymine ring mode (both expected near 1200 cm^{-1}) may accidentally have the same vibrational frequency of approximately 1193 cm^{-1}. As a consequence of this accidental degeneracy, the two modes may couple with one another and become split to yield coupled frequencies displaced about equally from the uncoupled value. Upon elimination of the wagging mode by C2′(*S*) deuteration, the coupling is eliminated and the localized thymine ring mode is revealed at 1194 cm^{-1} (Figure 6).

b. Ring Mode Near 950−970 cm^{-1}. The UVRR band of thymidine at 961 cm^{-1} is shifted to 954 cm^{-1} upon C2′(*S*) deuteration (Figure 6), a greater shift than expected for a localized base vibration. On the basis of the off-resonance Raman spectra (Figures 3 and 5), the unexpectedly large shift can be attributed to coupling of the thymine ring mode with a deformation of the C2′HD group of thymidine-(2′*S*)-*d*. Evidence of the C2′HD vibration is seen clearly near 990 cm^{-1} in the infrared spectrum and 993 cm^{-1} in the Raman spectrum of Figure 3. The effect upon base vibrations in the vicinity of 960 cm^{-1} is also apparent. For example, the 971-cm^{-1} thymine mode is lowered to 961 cm^{-1}.

c. Ring Mode at 789 cm^{-1}. The UVRR band of thymidine at 789 cm^{-1} (Figure 6) probably corresponds to the off-resonance Raman and infrared bands observed at 793 cm^{-1} (Figure 3). The band exhibits a remarkably large ^{13}C-isotope shift of −10 cm^{-1}.[18] The normal mode of vibration therefore involves both a significant contribution from the deoxyribose ring (to account for the ^{13}C shift) and a dominant contribution from the thymine base (to account for resonance at 244 nm). The observed C2′-deuteration effect is caused partly by coupling with the C2′−D deformation mode, as noted above.

d. Ring Mode at 757 cm^{-1}. This band is strong in the infrared (Figure 3), but has no apparent Raman counterpart. Such an intensity pattern is typical for an N−H out-of-plane bending vibration. Accordingly, the 757-cm^{-1} band is assigned to a vibration in which the thymine N3−H proton is displaced perpendicular to the plane of the base. This assignment is consistent with the absence of a significant C2′(*S*)-deuteration effect.

e. Ring Mode at 668 cm^{-1}. The 668 cm^{-1} band is weak but well resolved in the UVRR spectrum (Figure 6). Its counterpart in the off-resonance Raman spectrum is observed at nominally higher frequency, near 675 cm^{-1}. In each case a significant C2′-deuteration shift is observed, −7 cm^{-1} in the UVRR and −12 cm^{-1} in the Raman. The ^{13}C-isotope shift is also appreciable (−4 cm^{-1}). This mode is assigned to a delocalized thymine ring vibration in which the deoxyribose moiety makes a significant contribution. (See section 6.d, below.)

f. Ring Modes Below 600 cm^{-1}. The strong Raman band at 565 cm^{-1} exhibits a peculiarly large C2′(*S*)-deuteration shift of −18 cm^{-1}, which is close to the expected limiting value for a vibration localized in the C2′H_2 group.[19] In principle, the observed shift implies that the 565-cm^{-1} band originates from a vibration in which only the C2′H_2 group is in motion, while the remainder of the molecule is essentially fixed. This is consistent with the absence of any band near 565 cm^{-1} in the UVRR spectrum (Figure 6). However, assignment exclusively to the sugar C2′H_2 group is contradicted by the rather small ^{13}C-isotope shift of −5 cm^{-1} observed previously.[18] Further study is required to resolve the assignment of the 565-cm^{-1} band.

Another unexpectedly large C2′(*S*)-deuteration shift may occur for the Raman band at 474 cm^{-1} (Figure 4). Upon C2′-(*S*) deuteration, Raman intensity increases at 447 cm^{-1}, implying a shift of −27 cm^{-1}, well in excess of the expected limit for a vibration localized in the C2′H_2 group. A definitive assignment will require further analysis.

5. Correlation with Vibrational Spectra of Thymine. A detailed understanding of the vibrational coupling between thymine and deoxyribose moieties of thymidine would require complete vibrational analyses of both base and sugar residues. Toward this end, Aida et al.[20a] recently completed *ab initio* self-consistent-field molecular orbital calculations of the vibrational frequencies and intensities of thymine and thymine-1,3-d_2 and compared their results with experimental data, including polarized Raman spectra of a thymine single crystal.[20b] Unequivocal assignments have been reached for most of the infrared and Raman frequencies. Comparison of Raman spectra of thymine and thymidine shows that 22 of the Raman bands of the nucleoside correlate directly with those of the base. These are listed in Table 3, along with assignments based upon the thymine vibrational analysis of Aida et al.[20a] Similar frequency correlations can be tabulated for infrared spectra of thymidine and thymine (data not shown). However, the infrared intensities are more variable, presumably due to greater sensitivity of infrared transition moments to effects of intermolecular interactions which differ significantly in thymidine and thymine crystal lattices.

6. Some Examples of Vibrational Coupling between Thymine and Deoxyribose. a. Vibrational Modes Near 1430−1460 cm^{-1}. The two infrared bands of thymidine at 1435 and 1456 cm^{-1} exhibit nearly equal intensities. Conversely, in thymidine-(2′*S*)-*d* the lower frequency band is more intense than the higher frequency band. A qualitatively similar deuteration effect is observed for these band intensities in corresponding Raman spectra (Figure 2). One possible explanation for these effects is intramolecular vibrational coupling. However, because the 1435- and 1456-cm^{-1} bands are assigned to deformation vibrations of the exocyclic C5H_3 group,[20a] dependence upon deuterium substitution at the very remote C2′ site seems unlikely. An alternative explanation is *intermolecular* vibrational coupling, which can be rationalized as follows. The in-plane bending mode of the thymine N3−H group is expected in this region of the spectrum and is known to generate a broad infrared band near 1445 cm^{-1} which underlies the above noted bands due to C5H_3 deformations. The N3−H group is also an

(20) (a) Aida, M.; Kaneko, M.; Dupuis, M.; Ueda, T.; Ushizawa, K.; Ito, G.; Kumakura, A.; Tsuboi, M. *Spectrochim. Acta*. In press (1997). (b) Tsuboi, M.; Kumakura, A.; Aida, M.; Kaneko, M.; Dupuis, M.; Ushizawa, K.; Ueda, T. *Spectrochim. Acta*. In press (1997).

Table 3. Raman Bands Common to Thymidine and Thymine

thymidine		thymine		
cm^{-1}	int.[a]	cm^{-1}	int.[a]	assigned vibrational mode[b]
1692	w	1700	w	C2═O str
1665	s	1672	s	C4═O and C5═C6 in-phase str
1643	m	1652	w	C4═O and C5═C6 out-of-phase str
1483	m	1489	m	CH_3 degenerate def
1458	w	1459	w	CH_3 degenerate def
1437	w	1434	w	CH_3 sym def
1390	w	1408	m	ring [similar to benzene e_{2g} mode (1595 cm^{-1})]
1365	s	1368	s	ring + C6−H in-plane bend
1233, 1227	s	1258, 1246	m	C5−CH_3 stretch + ring [benzene b_{2u} (1308 cm^{-1})]
1199	s	1214	m	C5−CH_3 stretch + ring [benzene e_{2g} (1595 cm^{-1})]
1124	w	1156	m	ring [similar to benzene e_{1u} mode (1485 cm^{-1})]
1067	m	1048	w	CH_3 out-of-plane rock
1016	m	983	m	CH_3 in-plane rock
974	w	934	w	C6-H out-of-plane bend
794	m	804	m	ring [similar to benzene b_{1u} mode (1010 cm^{-1})]
772	s	741	s	pyrimidine ring breathing
675	s	616	s	in-phase C2═O and C4═O in-plane bend
565	s	556	m	ring [similar to benzene e_{2g} mode (605 cm^{-1})]
495	s	478	m	ring [similar to benzene e_{2g} mode (605 cm^{-1})]
379, 397	m	320	w	C5−CH_3 out-of-plane bend
297, 305	m	286	w	C5−CH_3 in-plane bend

[a] Relative Raman intensity (s strong, m medium, w weak) in the spectrum of crystalline powder. [b] Abbreviations: st stretch, def deformation.

intermolecular hydrogen bond donor to the C3′−O acceptor of a neighboring molecule in the thymidine crystal lattice.[17] Conceivably, 2′(*S*) deuteration could alter intermolecular vibrational coupling between the base of one thymidine molecule and the sugar of its neighboring molecule so as to lower the N3−H bending frequency by about 10 cm^{-1} (i.e. to ≈1435 cm^{-1}). This would have the effect of increasing the apparent intensity of the superimposed $C5H_3$ deformation band in the spectrum of the C2′(*S*) derivative.

b. The C2′(*S*)−D Bending Mode at 993 cm^{-1}. The Raman spectrum of thymidine exhibits two weak bands at 960 and 974 cm^{-1}. Either or both of these bands can be assigned to a vibration localized in the base residue of thymidine and specifically to a mode involving out-of-plane bending of the thymine C6−H group. The basis for this assignment is the fact that the C6−H out-of-plane bending mode of the free thymine base occurs at 934 cm^{-1},[20] and no candidate other than the 960- and 974-cm^{-1} bands exists for the corresponding mode in thymidine. As indicated in Section 4b., above, direct coupling occurs between the C2′(*S*)−D bending mode at 993 cm^{-1} and the thymidine vibration at 974 cm^{-1}. For the same reasons noted in Section 4b., C2′(*S*)−D bending is also coupled wth the thymidine 960-cm^{-1} mode. Thus, the 993 cm^{-1} band represents another example of vibrational coupling involving sugar and base moieties of thymidine. It is interesting to note that in crystalline thymidine the C6 proton of one molecule is very close to the C2′(*S*) proton of a neighboring molecule, involving a nonbonded H···H distance of 2.8 Å.[17] This suggests that the vibrational coupling of the 993-cm^{-1} mode may be mediated by an intermolecular H···D contact.

c. The C2′(*S*)−D Bending Mode Near 800 cm^{-1}. The pyrimidine ring of thymine is expected to exhibit strong and characteristic Raman bands near 750 and 800 cm^{-1}. The former is a quasisymmetrical ring breathing mode, while the latter is assignable to a triangle-like mode in which alternate pyrimidine bonds extend and contract in a concerted manner, similar to the b_{1u} mode (1010 cm^{-1}) of the benzene ring. These thymine markers are observed in the UVRR spectrum of thymidine (Figure 6) at 752 and 789 cm^{-1}, respectively, and are shifted by C2′(*S*) deuteration to 750 and 785 cm^{-1}. In off-resonance Raman spectra (Figure 3), where the markers occur at 772 and 794 cm^{-1} in thymidine and at 763 and 780 cm^{-1} in thymidine-C2′(*S*)-*d*, it is apparent that the shifts are caused by introduction of the C2′(*S*)−D bending mode at 801 cm^{-1}. Thus, the C2′(*S*)−D bending and thymine ring modes are vibrationally coupled. In this case, the intramolecular coupling involves groups which are separated by two covalent bonds (C2′−C1′−N1). The involvement of the deoxyribose moiety in the 794-cm^{-1} marker has also been demonstrated by the shift of −10 cm^{-1} observed upon ^{13}C substitution of the furanose carbons.[18]

d. Thymine In-Plane Ring Mode Near 600−700 cm^{-1}. Thymine exhibits a strong band at 616 cm^{-1} that has been assigned[20,21] to an in-plane bending mode in which the six-membered ring undergoes a skeletal deformation while the C2═O and C4═O groups simultaneously bend in-phase with one another (a "windshield-wiper" motion). In thymidine, this vibration is elevated to 675 cm^{-1} in the Raman spectrum and to 668 cm^{-1} in the UVRR spectrum (see section 4.e., above), possibly due to coupling with a deoxyribose mode at 632 cm^{-1}. In thymidine-1′,2′,3′,4′,5′-$^{13}C_5$, this mode is observed at 671 cm^{-1} in the Raman spectrum.[18] For thymidine-C2′-*d*, the corresponding mode occurs at 663 cm^{-1} in the Raman spectrum (Figure 3) and at 661 cm^{-1} in the UVRR spectrum (Figure 6). The C2′-deuteration shift of −12 cm^{-1} (675 → 663 cm^{-1}) in the Raman spectrum (or −7 cm^{-1} in the UVRR spectrum, 668 → 661 cm^{-1}) (Table 2) demonstates vibrational coupling with the deoxyribose moiety.

e. In-Plane Ring Deformations of Thymine. Thymine modes at 478 and 556 cm^{-1} have been assigned by Aida et al.[20a] to vibrations which are similar to the degenerate (e_{2g}) skeletal deformations of benzene at 606 cm^{-1}.[22−24] The corresponding modes occur in thymidine at 495 and 565 cm^{-1} (Figure 4), although they are not elevated to prominence in the UVRR spectrum (Figure 6). The higher frequency mode involves a significant displacement of the N1 atom, whereas the lower frequency mode does not.[20] This explains the more dramatic C2′ deuteration effect observed for the former (565

(21) Rush, T.; Peticolas, W. L. *J. Phys. Chem.* **1995**, *99*, 14647−14658.
(22) Wilson, E. B.; Decius, J. C.; Cross, P. C. *Molecular Vibrations*; McGraw-Hill: New York, 1955; p 259.
(23) Dollish, F. R.; Fateley, W. G.; Bentley, F. F. *Characteristic Raman Frequencies of Organic Compounds*; Wiley: New York, 1994; p 165.
(24) Tsuboi, M.; Nishimura, Y.; Hirakawa, A. Y.; Peticolas, W. L. *Biological Applications of Raman Spectroscopy*, Spiro, T. G., Ed.; Wiley: New York, 1984; Vol. 2, pp 109−179.

→ 547 cm^{-1}) than for the latter (495 → 492 cm^{-1}) (Figure 4). Qualitatively similar results are observed upon ^{13}C substitution.[18]

Summary and Conclusions

The present study has advanced significantly the assignment of bands in vibrational spectra of thymidine. In particular, definitive assignments have been reached for the methylene group vibrations of the deoxyribose moiety of thymidine. In combination with results from recent polarized Raman and vibrational analyses,[13,20] it has proved possible to characterize virtually all of the ≈30 vibrational modes of thymidine that are expected in the 200−1800 cm^{-1} interval.

Remarkably, the vast majority of bands in the vibrational spectra of thymidine are altered with C2′(*S*) deuteration. The observed changes in frequency and intensity indicate not only the effects of deuteration on vibrations localized at the C2′H_2 site but also the extensive vibrational coupling which exists between base and sugar moieties of thymidine. This is particularly evident in the difference spectrum of Figure 5. Many of the vibrational coupling interactions have been characterized in detail.

In pioneering work, Toyama et al.[12] demonstrated that UVRR spectra of C1′-deuterated ribonucleosides of adenine and guanine provided evidence of significant vibrational coupling between the purine bases and their ribosyl moieties. Although numerous frequency shifts were reported, the effects were less extensive than those observed here upon C2′(*S*) deuteration of thymidine. This can be attributed to the fact that the UVRR spectrum is informative only of resonance-enhanced vibrations (localized largely in the base), while the Raman and IR spectra are informative of virtually all normal modes of the nucleosides. The larger number of C2′-deuteration shifts observed in the off-resonance Raman than in the UV-resonance Raman spectrum of thymidine (cf. Figures 5 and 6) is consistent with this conclusion.

Further comparison of Figure 6 with the results of Toyama et al.[12] shows that UVRR perturbations in the C2′-deuterated pyrimidine nucleoside are smaller than those reported for the C1′-deuterated purine nucleosides. This could be due to fundamental differences in coupling of thymine and purine moieties with the attached sugar. Alternatively, the data may reflect attenuation of the effects of deuterium substitution at the more remote C2′ site. Preliminary studies of C2′-deuterated purine nucleosides favor the latter explanation (unpublished results of M. Tsuboi, M. P. Russell and G. J. Thomas, Jr.). Accordingly, deuteration at the glycosidic carbon (C1′) of thymidine would be expected to have an even greater effect on the vibrational spectra.

It is surprising that virtually all vibrational bands, including those above 1420 cm^{-1} and below 700 cm^{-1} in the off-resonance Raman spectrum of thymidine, are sensitive to C2′ deuteration. Toyama et al.[12] concluded, on the other hand, that bands outside the range 700−1420 cm^{-1} in adenosine and guanosine exhibited no coupling because they did not involve significant vibrational motions of the N9 atom and its immediate neighbors. Of course, these authors were concerned only with UV-resonance-enhanced vibrations of the bases. Our results show that vibrational coupling between deoxyribose and thymine moieties of thymidine extends to modes with vibrational frequencies greater than 1420 cm^{-1} and less than 700 cm^{-1}, and involves motions of exocyclic substituents of the thymine base including C2═O, N3-H, C4═O, C5-CH_3 and C6-H groups.

Table 4. Potential Markers of Thymidine and/or Furanose Conformation and Comparison with Raman Conformation Markers of Poly(dA-dT)[a]

			poly(dA-dT)[b]	
C2′H_2	C2′(*S*)D	$\delta(^2H)$	*B* form	*A* form
1230	1235	+5	1226	1239
1199	1194	−5		
1174				
1102				
898			893	872
794	780	−14	791	777
772	763	−9		
675	663	−12	665	642
632	626	−6		
565	547	−18	564	552
474	447	−27		

[a] Notation as in Tables 1 and 2. [b] Data from ref 6.

In addition to providing insight into the nature of vibrational coupling between thymine and deoxyribose moieties of thymidine, the present results identify Raman bands which may be exploited as markers of thymidine conformation in nucleic acids, thymine-containing drugs, and their complexes. These are listed in Table 4. Interestingly, several of the listed bands undergo C2′-deuteration shifts similar to those observed with the *B* to *A* conformational transition of poly(dA-dT).[6,7] For example, the band at 779 cm^{-1} was assigned tentatively to either thymine residues or the sugar−phosphate backbone of poly(dA-dT).[6] On the basis of the present results, the 779-cm^{-1} band of poly(dA-dT) can be assigned with confidence to thymine residues of the polynucleotide. Interestingly, our results show that the corresponding band in the thymine moiety (772 cm^{-1}) is brought into vibrational coupling with the C2′(*S*)−D sugar moiety by virtue of interaction with the C2′(*S*)−D bending mode at 801 cm^{-1}. Such knowledge should be useful in future studies to correlate the coupled mode with specific conformations of the deoxyribose ring of thymidine.

Acknowledgment. Support by the U.S. National Institutes of Health (Grant No. GM54378 to G.J.T.) is gratefully acknowledged. This paper is part LXII in the series Raman Spectral Studies of Nucleic Acids from the UMKC laboratory.

Supporting Information Available: IR and Raman spectra of thymidine and a listing of Raman frequencies and relative intensities of thymidine (5 pages). See any current masthead page for ordering and Internet access instructions.

JA962676T