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Vibrational Spectra and Experimental Assignments of Thymine and Nine of Its Isotopomers

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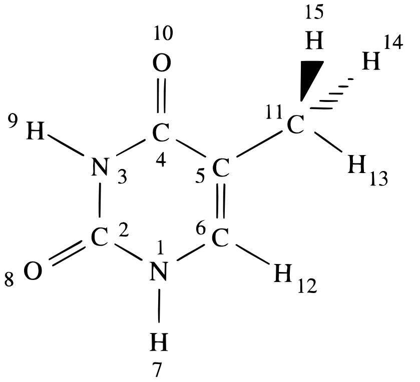
FT-IR and FT-Raman spectra of polycrystalline natural abundance thymine, nine of its isotopic derivatives, and 2-thiothymine (5-methyl-2-thiouracil) are reported from 50 to 3500 cm^{-1}. The isotopic derivatives are thymine-*6*-^{13}C, thymine-α-^{13}C, thymine-$^{15}N_2$, thymine-α,α,α,*6*-d_4, and their respective N-deuterated compounds. The vibrational spectra are assigned using the frequency shifts upon isotopic substitution and are compared to previous assignments, both experimental and ab initio based. Extensive mixing of the vibrations in the region below 1750 cm^{-1} complicates the vibrational assignments in this region. However, many of the exocyclic stretching and bending vibrations are assigned with confidence, and many of the couplings can be ascertained with these thymine derivatives. The spectra of the isotopic derivatives described here resolve many of the controversial assignments in the literature and correct some previously misassigned vibrational bands. This represents the most complete experimental vibrational study of thymine to date and provides a useful experimental basis for future theoretical calculations.

Introduction

Thymine (5-methyl-2,4-dioxopyrimidine, Chart 1) is one of the four nitrogenous bases found in DNA. It is one of the pyrimidine bases, along with cytosine, and hydrogen bonds to adenine in normal Watson−Crick base pairing. Thymine is one of the more important bases in the biologically important photoreactions of DNA; absorption of ultraviolet light by adjacent thymine molecules yields a cyclobutane photodimer[1−4] thought to have mutagenic and carcinogenic effects.[5] A full assignment of the vibrational spectra of the bases has become one of the essential steps in using vibrational spectroscopy to probe DNA structure and dynamics.[6−9] However, a complete understanding of the vibrational properties of many of the bases is still lacking, primarily as a result of the few complete experimental vibrational studies on isotopic derivatives of the nitrogenous bases.

Of all the bases, the vibrational spectrum of uracil (2,4-dioxopyrimidine) has been studied the most extensively with isotopic substitution, empirical normal coordinate analysis, and ab initio calculations.[7,10−16] The IR and Raman spectra of uracil in Ar matrix were examined for various deuterated uracils that include 5-*d*, 6-*d*, 5,6-d_2, 1,3-d_2, 1,3,5-d_3, 1,3,6-d_3, and d_4 derivatives.[12,13] Additionally, vibrational spectra have been reported for the 5,6-d_2, 1,3-d_2, and d_4 derivatives of crystalline uracil.[10] Despite these efforts, the assignments of all the out-of-plane vibrations and some in-plane vibrations remain controversial. Ab initio calculations have been performed in an attempt to sort out the disagreement in these assignments for uracil.[7] These calculations suggest NH and CH in-plane deformations are strongly coupled with many ring vibrations, and all of the vibrations involved are expected to be affected significantly by N- or C-deuteration.

CHART 1



Abbreviations for the Different Isotopic Derivatives

isotope	H_7, H_9 = H	H_7, H_9 = D
natural abundance	THNA	TDNA
$C_6 = {}^{13}C$	THC6	TDC6
$C_{11} = {}^{13}C$	THCα	TDCα
N_1, $N_3 = {}^{15}N$	THN2	TDN2
H_{12}, H_{13}, H_{14}, H_{15} = D	THD4	TDD4

In thymine, the substitution of a methyl group for a hydrogen atom at C_5 (Chart 1) is expected to significantly alter the normal mode description from that of uracil.[17] However, the effort devoted to the study of isotopically substituted thymine has not been as intense as that for uracil. One of the early papers studying the vibrational spectra of thymine is that of Susi and Ard,[18] who reported the IR and Raman spectra of polycrystalline natural abundance thymine and N-deuterated thymine. The observed frequencies were reported and studied, with the aid of a normal coordinate calculation, for only the in-plane vibrations. There are only a few recent studies of the vibrational properties of thymine in the solid (presumably noncrystalline powder) state,[19] in the polycrystalline state,[20,21] in Ar and N_2 matrixes,[22−24] and in the gas phase.[25] In the solid state,[19] the observed IR and Raman bands were assigned, but many

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fundamental bands were not observed due to the limited signal/noise of the reported spectra. In a recent study,[20] IR and Raman spectra of polycrystalline thymine are presented with reasonable signal quality. However, the bands are assigned based entirely on ab initio quantum mechanical and semiempirical calculations of the natural abundance thymine alone; no experimental isotopic data are reported to support the assignments. Also, the quantum mechanical calculation uses a minimum basis set.

More recent ab initio studies of thymine vibrations[24,26−28] have been performed with the use of much bigger basis sets and are expected to yield more accurate results. In one calculation,[26] ab initio MO and transform theory of resonance Raman scattering are used and the calculated frequencies are compared with the values observed from the spectrum of matrix-isolated thymine. In another study,[27] ab initio MO theory is used to calculate infrared and Raman band intensities and frequencies and they are compared with the observed infrared and Raman spectra of polycrystalline thymine. These two most recent ab initio studies of normal thymine and N-deuterated thymine provide important insights into the vibrational properties of thymine, particularly in the expected frequency sequence. However, they predict significantly different assignments and demonstrate the inconsistent results ab initio calculations can yield when there is a lack of experimental data.

Differences also exist between ab initio and semiempirical calculated assignments and their normal mode descriptions. For example, Susi and Ard[18] assigned the weak 1406 and strong 1447 cm^{-1} bands to an NH in-plane bending mode and the antisymmetric CH_3 deformation mode, respectively, on the basis of the experimental vibrational spectra and a normal coordinate calculation of two isotopomers. The same two experimental bands are calculated[26] to be the CH_3 scissors and the CH_3 deformation modes, respectively. The most recent ab initio study[27] assigns these modes to the same vibrations as Susi and Ard.

Because of these disagreements in the vibrational assignments for thymine, arising from the lack of available isotopic vibrational data, there is a need for data that can serve as the experimental basis for future theoretical calculations. In this paper, we describe IR and Raman spectra for 10 thymine isotopomers. These isotopomers include natural abundance thymine (THNA), thymine-*6*-^{13}C (THC6), thymine-α-^{13}C (THCα), thymine-$^{15}N_2$ (THN2), thymine-α,α,α,*6*-d_4 (THD4), and their respective N-deuterated compounds (TDNA, TDC6, TDCα, TDN2, TDD4, Chart 1). The IR and Raman spectra of 2-thiothymine (5-methyl-2-thiouracil) are also included in this study to help in the assignment of the carbonyl-related bands. Apart from the NH and CH stretching vibrations, which are relatively easy to assign, the observed band shifts are complex, indicating extensive coupling of internal coordinates. However, by using correlations between the observed band intensities and frequency shifts upon isotopic substitution, assignments are made for all of the observed modes and compared throughout with those reported previously for polycrystalline and matrix-isolated thymine and with ab initio results. As expected, the availability of vibrational spectra for several isotopomers resolves many of the conflicting band assignments.

Experimental Section

Natural abundance thymine (99%, Sigma), thymine-α,α,α,6-d_4 (99.4% D, CDN Isotopes), thymine-α-^{13}C (99% ^{13}C, Cambridge Isotope Laboratories), thymine-*6*-^{13}C (99% ^{13}C, Cambridge Isotope Laboratories), thymine-$^{15}N_2$ (98%+ ^{15}N, Cambridge Isotope Laboratories), and 2-thiothymine (98%, Aldrich) were obtained commercially. These six compounds were used as supplied. The corresponding N-deuterated thymines were obtained from the respective thymine derivative by using D/H exchange in D_2O. Briefly, 30−50 mg of an isotopomer was dissolved in approximately 15 mL D_2O, and the resulting solution was lyophilized until completely dry (∼30 h). This dissolving-drying procedure was repeated at least three times. Infrared spectra of the N-deuterated compounds showed no absorption at NH stretching frequencies, indicating that exchange of H for D was complete.

Infrared spectra were measured between 600 and 4000 cm^{-1} on an FT-IR instrument (Nicolet Magna-IR 750) equipped with a microscope (Nic-Plan). The optical bench and sample area were purged with dry nitrogen before and during the measurement. Spectra were obtained in transmission mode utilizing a microscope sample stage and an NaCl window. A minute amount of sample on the window was pressed and rolled over with a needle to an estimated thickness of 10 μm. The incident infrared beam was then focused on the polycrystalline sample to a diameter of approximately 110 μm. An interferogram was obtained by averaging 128 scans at a nominal resolution of 4 cm^{-1} and then Fourier-transformed into a single-beam spectrum with two levels of zero-filling (i.e. a zero-filling factor of 4), yielding an ordinate spacing of 0.96 cm^{-1}. A background spectrum was similarly recorded by moving the IR beam to a clean spot on the window. The ratio of the sample spectrum to the background gives a transmittance spectrum. Reported frequencies are accurate to about ±1 cm^{-1}. The spectra reported for THCα, THD4, and TDN2 are as measured, while the others have been slightly baseline-adjusted.

Infrared transmission spectra between 50 and 700 cm^{-1} were measured on polyethylene (PE)/sample pellets using the above nitrogen-purged Nicolet system. For these spectra, the FT-IR instrument was fitted with a Nicolet Solid Substrate far-IR beam splitter and a DTGS detector. Instrumental conditions were similar to those described above for the mid-infrared. The background spectrum was recorded with an empty sample compartment. Some spectra display strong interference bands below 350 cm^{-1} from water vapor, due to incomplete purging of the instrument and incomplete subtraction of the water bands. The water bands are especially troublesome when sample bands are weak. In these cases, a second spectrum was recorded with a higher sample concentration to improve the reliability of the observed sample bands.

FT-Raman spectra were measured with an FT-IR spectrometer (Bruker IFS 88) equipped with a Raman attachment (FRA 106). Excitation was provided by an air-cooled, diode-pumped Nd:YAG laser ($\lambda = 1.064$ μm) with a power of 300 mW. The polycrystalline samples were pressed into aluminum cups that were about 2 mm in diameter and 1 mm deep. Spectra were obtained using a 180° (backscattering) geometry. Double-sided interferograms were recorded at a nominal resolution of 4 cm^{-1} under a correlation test mode that checks all interferogram points. One level of zero-filling was used prior to the transform, yielding spectra with an ordinate spacing of 1.92 cm^{-1}. Accuracies of reported frequencies are better than ±2 cm^{-1}. The spectra of the N-deuterated thymines were obtained by averaging 10 100-scan spectral files, and the spectra of other samples were usually averages of four files, with each 100-scan spectrum requiring 2.5 min. All spectra were corrected with an instrument response function derived from the spectrum of a calibration lamp. To check whether the FT-Raman spectra obtained with this instrument contain artifacts in the region

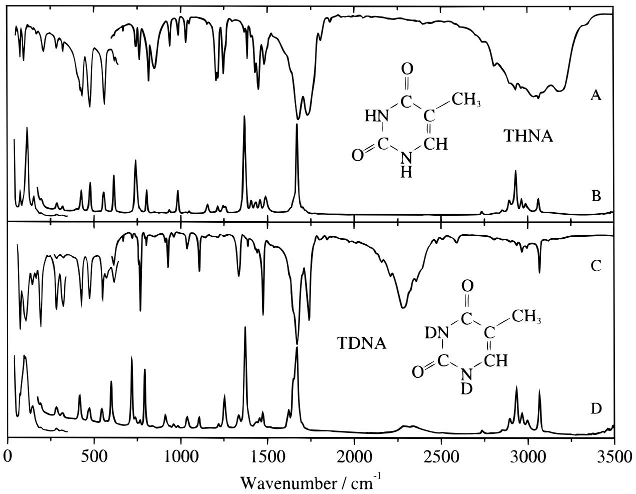

Figure 1. FT-IR transmittance (A, C) and FT-Raman (B, D) spectra of natural abundance polycrystalline thymine (THNA) and its N-deuterated derivative (TDNA). Insets in the low-wavenumber region of the FT-IR spectra were measured at different concentrations in polyethylene pellets. The inset in the low-wavenumber region of the Raman spectrum is rescaled and offset for clarity.

below 150 cm^{-1}, low-frequency Raman spectra of THNA, THD4, THN2, TDN2, and 2-thiothymine were also obtained on a dispersive Raman system (SPEX 1403) using either 488 or 514.5 nm Ar^{+} laser excitation and standard photon-counting electronics. No significant differences were found when comparing data from two instruments, indicating that the features observed in the FT-Raman spectra are genuine.

Peaks in the FT-IR and FT-Raman spectra were tabulated using the peak-picking function of the Spectra Calc software package (Galactic Industries Corporation, Salem, NH). The peak wavenumbers were then checked individually by expanding the spectrum and examining the listed band positions. Bands that appear as shoulders were manually added to the tables.

To be consistent and avoid confusion, IR frequencies are used throughout unless otherwise specified. Following convention, the in-plane deformation vibrations of NH, CO, and $C-CH_3$ are denoted bending vibrations (bends), and the out-of-plane deformations of these bonds are denoted wagging vibrations (wags). For the methyl group vibrations, HCH angle change modes are all referred to as CH_3 deformations and CCH angle changes are separated into rock (in-plane) and wag (out-of-plane). A ring deformation is strictly defined as ring angle changes in the ring plane only, while a ring torsion is defined as the out-of-plane motion of the ring atoms.

Results

Figure 1 shows the FT-Raman and FT-IR transmittance spectra of THNA and its N-deuterated counterpart, TDNA. The IR and Raman spectra of the other isotopomers are depicted in Figures 2−5. Figure 6 shows the IR and Raman spectra of 2-thiothymine. The two curves for each of the far-IR spectra of TDNA, TDC6, THCα, and TDCα were obtained with different sample concentrations, which was found to be necessary to observe the weaker thymine bands without interference from atmospheric water bands. The sharp, weak IR feature at 668 cm^{-1} (curves A and C in Figures 1−6) is the CO_2 bending band, while the features at about 2340 and 2360 cm^{-1} are the associated stretches. These bands present no interference for 2-thiothymine and the N-protonated compounds, which have no fundamental bands in this region. For the N-deuterated compounds, however, these CO_2 stretching bands are on the high-wavenumber side of the CD and broad ND stretching vibrational bands. Subtraction of a CO_2 spectrum only changes the positions and apparent intensities of some bands in this region; it does not eliminate any features. Thus, we believe the bands in this region all originate from overtone and combination vibrations of thymine.

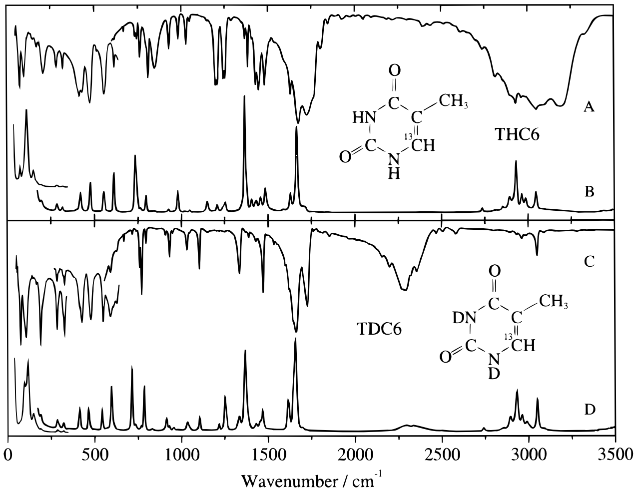

Figure 2. FT-IR transmittance (A, C) and FT-Raman (B, D) spectra of polycrystalline thymine-*6-*^{13}*C* (THC6) and its N-deuterated derivative (TDC6) as per Figure 1.

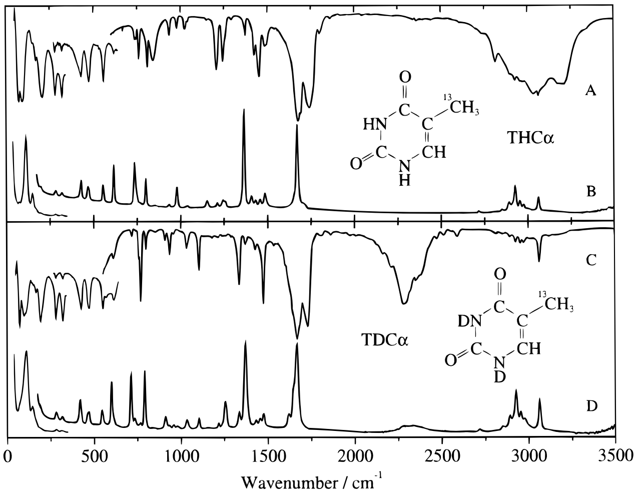

Figure 3. FT-IR transmittance (A, C) and FT-Raman (B, D) spectra of polycrystalline thymine-α-^{13}*C* (THCα) and its N-deuterated derivative (TDCα) as per Figure 1.

In general, all the spectra in Figures 1−6 exhibit excellent signal/noise, and many bands are easily seen on the plotted scales. The band positions so identified in the IR and Raman spectra are collected in Table 1 for the isotopic derivatives of thymine. As seen in Figures 1−5 and Table 1, many vibrational bands are shifted upon a single isotopic substitution, suggesting extensive mixing among the internal coordinates. For example, substitution of ^{13}C at C_{11} (THCα) yields frequency shifts of greater than 3 cm^{-1} in more than 20 vibrations, while this atom is expected to be directly involved in no more than 11 modes.

The spectra reported here for natural abundance thymine are comparable to those reported previously.[18,20,21,27] N-deuterated thymine (TDNA) is the only isotopic derivative of thymine whose vibrational spectrum has been reported previously.[18,22,27] Direct comparison of our spectra with those in the former two studies is not very meaningful because of the different molecular

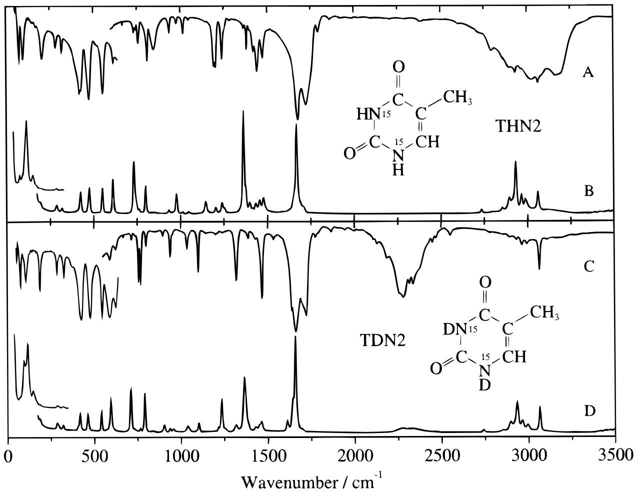

Figure 4. FT-IR transmittance (A, C) and FT-Raman (B, D) spectra of polycrystalline thymine-$^{15}N_2$ (THN2) and its N-deuterated derivative (TDN2) as per Figure 1.

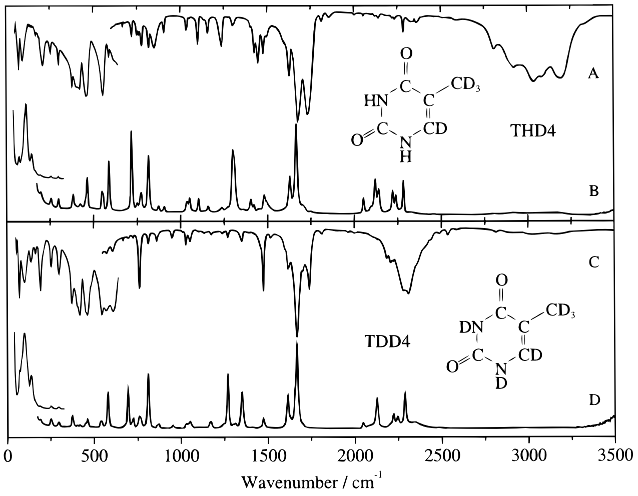

Figure 5. FT-IR transmittance (A, C) and FT-Raman (B, D) spectra of polycrystalline thymine-α,α,α,*6*-d_4 (THD4) and its N-deuterated derivative (TDD4) as per Figure 1.

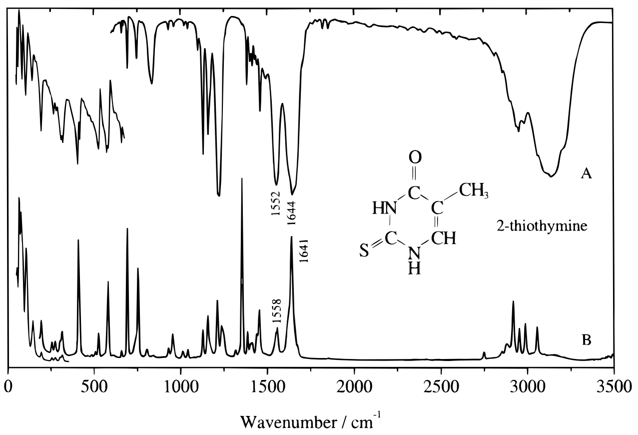

Figure 6. FT-IR transmittance (A) and FT-Raman (B) spectra of polycrystalline 2-thiothymine. The inset in the low-wavenumber region of the FT-IR spectrum was measured in a polyethylene pellet. The inset in the low-wavenumber region of the Raman spectrum is rescaled and offset for clarity. Only the relevant band positions used for assignment are given.

environments in the three studies, which is expected to significantly shift the mode frequencies (vide infra). Susi and Ard[18] studied thymine in mulls, while Nowak's spectra [22] were recorded on thymine in Ar matrix. Only Aida et al.[27] reported spectra of N-deuterated thymine as a polycrystalline powder. A general agreement in band frequencies is observed between our study and that by Aida et al.[27]

Discussion

For the 15-atom, planar thymine molecule (Chart 1), 39 fundamental modes are expected, of which 26 are in-plane and 13 are out-of-plane modes. The six stretching vibrations involving the hydrogen atoms are expected at highest wavenumbers between 2800 and 3200 cm^{-1}. The carbonyl stretches should be near 1700 cm^{-1}, with a ring stretch involving the C=C double bond at a similar frequency. The other five ring stretches and the $C-CH_3$ stretch lie over a wide frequency range between 700 and 1500 cm^{-1}. There are another six ring vibrations involving angle changes, three in-plane (deformation) and three out-of-plane (torsion). The exocyclic deformation modes can be separated into two types of vibrations, bend and wag, for each of the two N−H, two C−O, C_6-H, and C_5-C_{11} bonds. For the methyl group, besides the three stretches, there are five modes involving CCH and HCH angle changes. The last vibration is the methyl torsion, the rotation of the methyl group about the C_5-C_{11} bond. A detailed discussion of the experimental assignment of these modes in thymine based on the isotopic vibrational spectra recorded here is given below and summarized in Table 1.

NH Stretches. The NH stretching vibrations are found as a doublet at 3179 and 3195 cm^{-1} in the IR spectrum of THNA, much lower than previously observed at 3434 and 3480 cm^{-1} in Ar or N_2 matrix[22−24] and at 3437 and 3484 cm^{-1} in gaseous thymine.[25] This difference is attributed to stronger intermolecular hydrogen bonding in the polycrystalline state, which is expected to lower the stretching frequency. These strong bands are shifted to lower frequency by about 900 cm^{-1} upon N-deuteration. Isotopic substitution with ^{15}N on both N atoms shifts them to 3165 and 3186 cm^{-1} for THN2, and to 2259 and 2280 cm^{-1} from 2278 and 2291 cm^{-1}, respectively, for TDN2. Calculated frequencies are 3171 and 3188 cm^{-1}, and 2268 and 2281 cm^{-1}, for the THN2 and TDN2 isotopomers, respectively, if an isolated harmonic oscillator is assumed. The small differences between the observed frequency shifts and those predicted from a simple diatomic model indicate that these normal vibrations are highly localized NH(D) stretches.

In the Raman spectra, no NH stretching bands are observed (curves B in Figures 1−5), but weak, broad doublet bands are seen at ca. 2290 and 2340 cm^{-1} in the spectra of the N-deuterated derivatives (curves D). Specifically, the ND stretches are observed at 2289 and 2337 cm^{-1} in TDNA and are further shifted to 2277 and 2329 cm^{-1} in the $^{15}N_2$, $^2H_2$ isotopomer (TDN2). We attribute this doublet in the Raman spectra of the N-deuterated isotopomers to the two ND stretches.

For the NH stretches, only Mathlouthi et al.[19] reported the feature at about 3200 cm^{-1} in polycrystalline thymine. In the molecular structure of thymine (Chart 1), there is a carbonyl group on both sides of the N_3H bond to withdraw electrons. As a result, the N_3H bond is slightly weaker than the N_1H bond, and therefore, the N_1H bond stretch is assigned at the higher frequency. This result is supported by ab initio calculations[24,26,27] and is likely the same in polycrystalline thymine. Thus, we assign the 3195 cm^{-1} band to the N_1H stretch and the 3179 cm^{-1} band to the N_3H stretch.

CH Stretches. The infrared spectra of N-protonated isotopic derivatives suffer from interference from a broad, intense

TABLE 1: Frequencies (cm^{-1}) and Assignments of the Observed Infrared (IR) and Raman (R) Bands for 10 Isotopic Derivatives of Thymine[a]

THNA		THC6		THCα		THN2		THD4		TDNA		TDC6		TDCα		TDN2		TDD4		
IR	R	IR	R	IR	R	IR	R	IR	R	IR	R	IR	R	IR	R	IR	R	IR	R	assignment[b]
3195		3192		3207		3186		3195		2291	2337	2291	2335	2293	2336	2280	2329	2310	2343	$\nu(\mathrm{N_1H})$
3179		3175		3177		3165		3177	3172	2278	2289	2275	2292	2280	2283	2259	2277	2280		$\nu(\mathrm{N_3H})$
3062	3062	3048	3047	3062	3060	3061	3060	2284	2283	3067	3066	3051	3050	3062	3062	3065	3064	2292	2289	$\nu(\mathrm{C_6H})$
2985	2987	2983	2987	2971	2975		2987	2236	2238	2994	2996	2989	2991	2974	2975	2993	2995		2248	$\nu^a(\mathrm{CH_3})$
2963	2964	2962	2964	2955	2954	2963	2964	2222	2221	2966	2966	2962	2964	2953	2954	2963	2962	2223	2223	$\nu^a(\mathrm{CH_3})$
2930	2931	2930	2931	2927	2927	2930	2931	2142	2140	2936	2935	2932	2933	2926	2927	2932	2933	2131	2127	$\nu^s(\mathrm{CH_3})$
1731	1720	1724	1712	1740		1721		1732		1739		1726		1728		1721		1740		$\nu(\mathrm{C_2O})$
1678	1670	1675	1666	1676	1670	1675	1668	1675	1666	1671	1670	1660	1658	1668	1668	1659	1656	1667	1666	$\nu(\mathrm{C_4O})$
1678	1670	1629	1629	1676	1670	1675	1668	1628	1629	1626	1623	1620	1614	1642	1648	1637	1643	1615	1614	$\nu(\mathrm{C{=}C})$
1482	1488	1480	1484	1485	1484	1473	1477	1476	1480	1474	1473	1471	1467	1473	1475	1467	1463	1474	1473	ν(ring)
1447		1446		1452		1442		1447	1448	1107	1106	1103	1104	1103	1102	1100	1101	1099		$\delta(\mathrm{N_1H})$
1428	1432	1428	1432	1423	1432	1419	1434	1099	1102	1431	1428	1428	1430	1427	1432	1430	1430	1045	1043	$\delta^a(\mathrm{CH_3})$
1406	1405	1406	1405	1406	1407	1399	1399	1404	1403	908	910	906	913	907	910	896	902			$\delta(\mathrm{N_3H})$
1383		1383	1376	1373		1382	1376	1053	1052	1387		1388		1377		1388	1386	1053	1052	$\delta^a(\mathrm{CH_3})$
1366	1367	1365	1365	1365	1365	1363	1363	1135		1377	1372	1369	1367	1370	1371	1367	1365	1133		$\delta(\mathrm{C_6H})$
1366	1367	1365	1365	1365	1365	1363	1363	1016		1377	1372	1369	1367	1370	1371	1367	1365	1011		$\delta^s(\mathrm{CH_3})$
1257	1259	1254	1253	1255	1257	1254	1253	1300	1299	1334	1334	1335	1332	1335	1334	1318	1317	1350	1351	ν(ring)
1246	1245	1242	1241	1244	1245	1240	1239	1237	1237	1253	1253	1250	1251	1255	1255	1238	1234	1270	1268	ν(ring)
1215	1214	1208	1207	1207	1210	1202	1203	1156	1158	1212	1218	1217	1218	1215	1216	1211	1214	1175	1170	$\nu(\mathrm{C{-}CH_3})$
1152	1155	1148	1149	1149	1153	1144	1145		873	1141	1153	1149		1148	1153	1146			871	r$(\mathrm{CH_3})$
1048	1048	1047	1048	1039	1041	1047	1048	459	452	1045		1046	1045	1040		1048		455	451	$\gamma(\mathrm{CH_3})$
1030	1025	1028	1025	1023	1029	1015	1012	1038	1037	1037	1037	1031	1033	1032	1033	1036	1039	1029	1029	δ, ν(ring)
984	983	982	979	978	979	978	977	904	906	741	738	740	732	741	732	737	728	725	724	γ(NH)
935	933	928	925	934	933	935	933	379	383	925	935	931	929	934	933	938	937	374	375	$\gamma(\mathrm{C_6H})$
847	850	846	842	841	852	846	844	849		850		852		855		851		861		$\gamma(\mathrm{C_2O})$
847	850	846	842	841	852	846	844	849		841	832	829	831	843	834		838			$\gamma(\mathrm{C_4O})$
814	804	809	796	810	800	810	800	817	814	794	792	795	784	799	790	798	790	811	810	ν, δ(ring)
761	765	761	765	760	763	758	761	776	771	768	767	770	769	768	767	768	765	761	759	τ(ring)
741	740	733	734	737	736	732	732	719	717	720	717	716	713	718	713	715	711	695	694	ν(ring)
741	740	744		744		743	743	759		616	615	608		614	615	625		610		γ(NH)
617	615	614	613	617	615	614	611	589	588	590	599	591	597	599	599	589	595	571	580	δ(CO)
559	557	556	553	559	553	553	551	555	553	551	545	549	541	552	545	546	541	547	543	δ(ring)
475	478	475	478	475	468	474	476	464	464	474	474	479	464	471	470	477	462	463	462	δ(ring)
432	427	430	422	430	427	427	425	421	424	427		427		426	420	425	418	419	420	τ(ring)
403	399	402	400	407	402	402		406	398	408	418	416	414					407	404	δ(CO)
321	319	320	317	319	317	318	317	302	298	322	319	328	321	321	316	326	319	301	296	$\gamma(\mathrm{C{-}CH_3})$
284	287	283	287	280	283	283	287	255	254	283	283	285	285	281	281	285	285	254	252	$\delta(\mathrm{C{-}CH_3})$
209	196	209	196	207	194	204	192	210	195	192	190	191	190	193	188	188	186	191	190	τ(ring)
	153		153		150		150		145	144	144		149		146		148	137	138	$\tau(\mathrm{CH_3})$

[a] Bands that were not observed are indicated with empty entries. The table is arranged in wavenumber descending order for the natural abundance thymine, and the frequencies of the same vibration for the other isotopomers are entered in the same row. [b] Abbreviations: ν, stretching; δ, deformation; γ, wag (out-of-plane deformation); r, in-plane rock; τ, torsion; superscripts s and a denote symmetric and asymmetric, respectively.

background absorption in the 3000 cm^{-1} vicinity (curves A in Figures 1−5). This broad background is believed to originate from overtone and combination vibrations, whose intensities are enhanced through Fermi resonance. Because of this background, the Raman spectra are used for assignment of the CH stretching vibrations. The Raman band at ca. 3062 cm^{-1} (THNA) is the highest frequency band in the region and can be assigned to the C_6H stretching mode. This 3062 cm^{-1} band is the only band in this region that downshifts to 3047 cm^{-1} upon ^{13}C substitution at C_6 (THC6, Table 1), consistent with the calculated shift assuming an isolated CH diatomic oscillator. The simple frequency shift in THC6 and insensitivity to other isotopic substitution suggest a nearly pure CH stretch normal mode.

An examination of the Raman band intensities immediately to lower wavenumber from the C_6H stretching band reveals that a band at 2931 cm^{-1} is always the strongest, and more intense than the 2894 cm^{-1} band, in the relevant spectra (curves B and D in Figures 1−4). It is assigned to the symmetric CH_3 stretch because this stretch normally has a stronger Raman intensity than the asymmetric CH_3 stretches. Two asymmetric CH_3 stretches are expected at higher frequencies. Therefore, we assign the 2964 and 2987 cm^{-1} bands to the asymmetric CH_3 stretches, which belong to different symmetries (one in-plane and one out-of-plane). These assignments are supported by the isotopic shifts upon ^{13}C substitution at C_{11} (THCα, Table 1). The 2931, 2964, and 2987 cm^{-1} bands in THNA are downshifted by 4, 10, and 12 cm^{-1} to 2927, 2954, and 2975 cm^{-1} in THCα, respectively, while there is hardly any shift upon ^{13}C substitution at C_6 (THC6) and ^{15}N substitution (THN2). When the methyl group is deuterated (THD4), all the CH_3 stretches are shifted to around 2200 cm^{-1}, as expected.

Of all the previous assignments, the assignment of these CH stretching bands are consistent only with that of Susi and Ard.[18] The assignment[19] of the 2894 and 2815 cm^{-1} bands to asymmetric CH_3 stretch and C_6H stretch, respectively, are incorrect; the assignment of these modes to overtones of the 1447 and 1406 cm^{-1} bands, respectively, is more consistent with the lack of observed shift upon C_6 or C_{11} substitution by ^{13}C. Assignment of the C_6H stretching mode to the band at 2992 cm^{-1} in Ar matrix-isolated thymine,[22−24] about 70 cm^{-1} lower than the band assigned here, is also inconsistent with the isotopic shifts observed in this study. One possible explanation for this difference in assignments is that because all the CH stretching bands are weak, the 2992 cm^{-1} band observed in Ar matrix-isolated thymine may be assigned to a methyl stretch and the band for the C_6H stretch is, in fact, a weak feature found at ca. 3070 cm^{-1} (Figure 5, ref 23) whose frequency is not reported. This reassignment of the observed matrix-isolated thymine

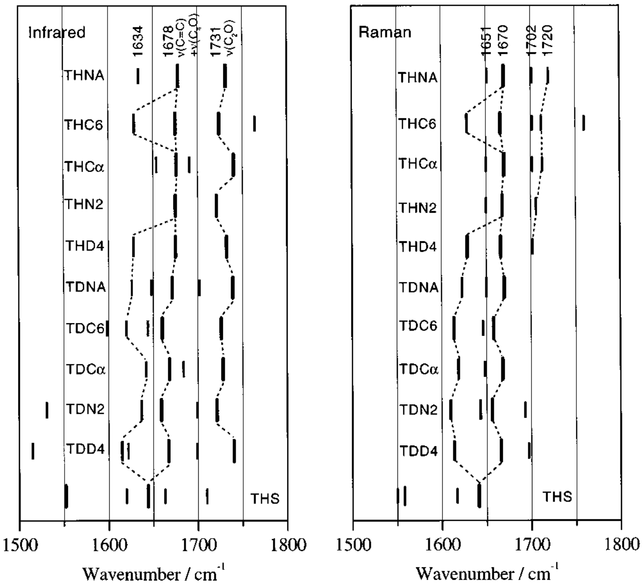

Figure 7. IR and Raman correlation diagram for the C_2O, C_4O, and C=C stretching bands. THS stands for 2-thiothymine. The thickness of the solid lines represents the intensity, while the dotted lines indicate the correlation among the observed bands upon isotopic substitution.

vibrations resolves all of the inconsistencies in the reported C_6H and methyl CH stretches.

CO and CC Double-Bond Stretches. From the correlation diagram in Figure 7, the C_2O stretch can be confidently assigned to a band at 1731 cm^{-1} in the IR. This strong IR band in THNA disappears in 2-thiothymine, while a new band appears at 1552 cm^{-1}, and the strong band at 1678 cm^{-1} shifts to 1644 cm^{-1} (Figure 6). The shift of the IR band from 1731 to 1552 cm^{-1} is expected for a local C_2O stretch upon replacement of O by S. Similarly, the Raman band at 1670 cm^{-1} in THNA is shifted to 1641 cm^{-1} in 2-thiothymine and a new medium-intensity band appears at 1558 cm^{-1}. The stronger Raman intensity of the C_2S stretching mode at 1558 cm^{-1} compared to the C_2O mode reflects the greater polarizability of the $C_2{=}S$ bond than that of the $C_2{=}O$. Because the frequency of this band varies in a complicated manner with isotopic substitution, this mode also has contributions from other internal coordinates. For example, the downshift of this band in THC6 indicates some contribution of the C=C stretch to this band, while shifts upon isotopic substitution at NH are more complex, indicating mixing with the NH in-plane bending vibrations and possible coupling between the two CO stretches.

We propose that the intense IR band at 1678 cm^{-1} in THNA actually arises from the accidentally degenerate C_4O and C=C stretches. Examination of the spectral patterns of the THC6 derivative in Figure 7 shows an intense band at 1630 cm^{-1} in both IR and Raman spectra, which may be the C=C stretch component separated from the 1678 cm^{-1} band. The existence of a similar band in THD4 indicates some involvement of the C_6H bend in the 1678 cm^{-1} band of the other N-protonated species. A medium-intensity Raman band at about 1620 cm^{-1} is observed for all the N-deuterated isotopic derivatives, which we attribute to the C=C stretch; it is shifted down from 1670 cm^{-1} in the N-protonated derivatives because coupling with the NH bends, which normally pushes the C=C stretch to higher frequency, is removed upon N-deuteration. Therefore, we suspect the C=C and C_4O stretches are accidentally degenerate at 1678 cm^{-1} in natural abundance thymine. This assignment is also consistent with the intensities observed in the IR and Raman spectra in this region. Most of the IR intensity of the 1678 cm^{-1} band arises from the C_4O stretch. Because the Raman intensity of the C_4O and C_2O stretches are expected to be similar and the C_2O stretch gives a very weak Raman band, the Raman intensity of the intense 1670 cm^{-1} band must contain a contribution from the C=C stretch. An alternative explanation is that the stronger Raman intensity in the 1678 cm^{-1} band may arise from an in-phase combination of the two C=O stretches, while the 1731 cm^{-1} band is the out-of-phase combination. The shift of the 1678 cm^{-1} band to 1644 cm^{-1} in 2-thiothymine implies some contribution of C_2O to the 1678 cm^{-1} band.

Our assignment of the C_2O stretch as the 1731 cm^{-1} band and the C=C stretch as part of the 1678 cm^{-1} band is consistent with those made previously on thymine in Ar and N_2 matrixes[23] and in the polycrystalline state.[18-20] The assignment of the 1678 cm^{-1} band to both C_4O and C=C stretches is supported by an observed frequency shift and an intensity change in the recently reported IR spectrum of 4-thiothymidine.[29] However, the assignment of the C_4O vibration has been controversial; the C_4O vibration has been previously assigned to all possible bands in this region. In Ar matrix-isolated thymine, the assignment of the 1712 cm^{-1} band group to the C_4O stretching mode and that of the 1684 cm^{-1} band group to the C=C mode seems to be agreed upon.[22-24] It should be pointed out that two recent ab initio studies give quite different normal mode descriptions for the observed bands in this region. In one study,[26] the three normal modes of the natural abundance thymine are calculated, from high to low wavenumber, to be dominated by localized C_2O, C_4O, and C=C stretches, respectively. The other study[27] also has a nearly pure C_2O stretch as the highest wavenumber mode, but the other two are the in-phase and out-of-phase vibrations of the C_4O and C=C stretches. More specific isotopic derivatives at C_2O, C_4O, and C_5C_6 are needed to experimentally distinguish these two possible assignments.

NH In-Plane Bends. The NH bending modes of all the exocyclic in-plane modes are expected to appear in the 1400−1500 cm^{-1} region. Of all the observed bands in this region, only two, at 1406 and 1447 cm^{-1} (THNA) in the IR spectrum, exhibit a downshift upon ^{15}N substitution (Table 1). These modes also shift to 1100 and 900 cm^{-1} in TDNA upon N-deuteration (Table 1). We therefore assign these two modes to the NH bends. The above assignments are in agreement with Susi and Ard[18] for the 1406 cm^{-1} band and with Florián and Hrouda[20] for the 1447 cm^{-1} band. The previous assignments[19] of the 1482 and 1492 cm^{-1} bands to the NH bends are inconsistent with the observed frequency shifts in the N-deuterated derivatives. The assignment of the 1482 cm^{-1} band by Florián and Hrouda[20] and the 1492 cm^{-1} band by Susi and Ard[18] to the NH in-plane bend are likewise inconsistent with the frequency shift observed here; the 1482 cm^{-1} band is observed in all the isotopomers and is insensitive to either ^{15}N substitution or N-deuteration. Additionally, a shoulder is clearly observed at 1496 cm^{-1}, corresponding to the 1492 cm^{-1} mode previously assigned to an NH bend, in only some of the IR spectra reported here, which we tentatively assign to 761 + 740. Note that in Ar matrix,[20,26] the high-wavenumber NH bend is assigned at 1472 cm^{-1}, significantly higher than 1447 cm^{-1} assigned here, while the low-wavenumber NH bend is assigned between 1405 and 1221 cm^{-1}, lower than our 1406 cm^{-1} band. These frequency differences are expected from different hydrogen bonding in Ar matrix and the polycrystalline solid, as discussed above.

CH_3 Deformations and Rocks. Five vibrations in the methyl group are associated with the CCH and HCH angle changes: three deformations, one rock, and one wag. Three IR bands at

1366, 1383, and 1428 cm^{-1} are observed in THNA and can be assigned to the three CH_3 deformation modes. Substitution with ^{13}C on the methyl group (THCα) shifts these bands to lower wavenumbers by 1, 10, and 5 cm^{-1}, respectively. When the methyl group is deuterated, these bands shift to 1016, 1053, and 1099 cm^{-1}, respectively, in THD4, and 1011, 1053, and 1045 cm^{-1}, respectively, in TDD4. The anomalously low frequency of the 1045 cm^{-1} asymmetric CD_3 deformation in TDD4 is a result of the loss of coupling with the NH wag, which has shifted down to 725 cm^{-1} from 984 cm^{-1} upon N-deuteration (vide infra).

Previous assignments in this region of the spectrum have been controversial. In the most recent ab initio study, the bands in this region, attributable to two NH bending and three CH_3 deformation vibrations, were not assigned, even though the calculation provided a frequency sequence for these vibrations.[27] The asymmetric CH_3 deformation bands in Ar matrix-isolated thymine are assigned at higher wavenumbers between 1455 and 1433 cm^{-1}, while the assignments of the bands at 1388 and 1357 cm^{-1} are unclear.[22-24,26] Other researchers[18,19] have correlated the 1447 cm^{-1} IR band to the 1457 cm^{-1} Raman band and assigned them to one of the asymmetric CH_3 deformations. This correlation and assignment are inconsistent with our spectra, which show the Raman band at a higher frequency than the IR band by 10 cm^{-1} or more, and are observed in all but the TDD4 spectrum. More importantly, the 1457 cm^{-1} Raman band is insensitive to isotopic substitution, while the 1447 cm^{-1} IR band is not observed for the N-deuterated isotopomers even though the band has a consistent medium intensity for all the N-protonated species. In the discussion above, the 1447 cm^{-1} IR band has been attributed to one of the NH in-plane bending modes, more consistent with the observed frequency shifts upon isotopic substitution. The assignment of the two low-wavenumber CH_3 deformation bands is in agreement with that of Florián and Hrouda,[20] but their assignment of a 1468 cm^{-1} Raman band (not observed in the IR) to an asymmetric CH_3 mode is different from our assignment of this vibration to the 1428 cm^{-1} band. In fact, a band at 1468 cm^{-1} is not observed in our Raman spectrum of THNA.

The other two CH_3 angle change related bands can be assigned at 1152 cm^{-1} for the rock (in-plane) and 1048 cm^{-1} for the wag (out-of-plane). Shifts of 3 and 9 cm^{-1} are observed for these two vibrations, respectively, upon ^{13}C isotopic substitution on the exocyclic methyl carbon. The assignment[18,20] of the 984 cm^{-1} band to the CH_3 rock is not correct because this band is not observed in the IR spectra of any of the N-deuterated species.

CO Bends. Two in-plane and two out-of-plane bending bands are expected for the two C=O bonds. A correlation diagram for these modes is shown in Figure 8 for the IR. The 847 cm^{-1} band is broad and its intensity is weak to moderate in the IR spectrum of THNA, characteristic of a hydrogen-bonded CO wag. The 847 cm^{-1} band appears as a single band in the IR spectra of the N-protonated species, but in TDNA, TDCα, and TDC6, two bands split by 10−20 cm^{-1} are seen. Previous ab initio calculations[24,26] suggested that the frequencies of the two CO wags are similar. Also, these vibrations in Ar matrix are assigned[22-24] at 754 and 764 cm^{-1}, two bands that are only 10 cm^{-1} apart. Therefore, we assign the 847 cm^{-1} band in THNA to the two CO wags. A significant difference in frequency is observed between Ar matrix-isolated thymine and polycrystalline thymine, readily attributable to increased hydrogen bonding in the latter. Our assignment of the CO wag is consistent with that of Florián and Hrouda,[20] but different from that of Mathlouthi et al.,[19] who assigned the 847 cm^{-1} band to an NH wag and did not observe any CO wagging band. The assignment of the 847 cm^{-1} band to an NH wag is inconsistent with an observed band at a similar frequency in the spectra of the N-deuterated species.

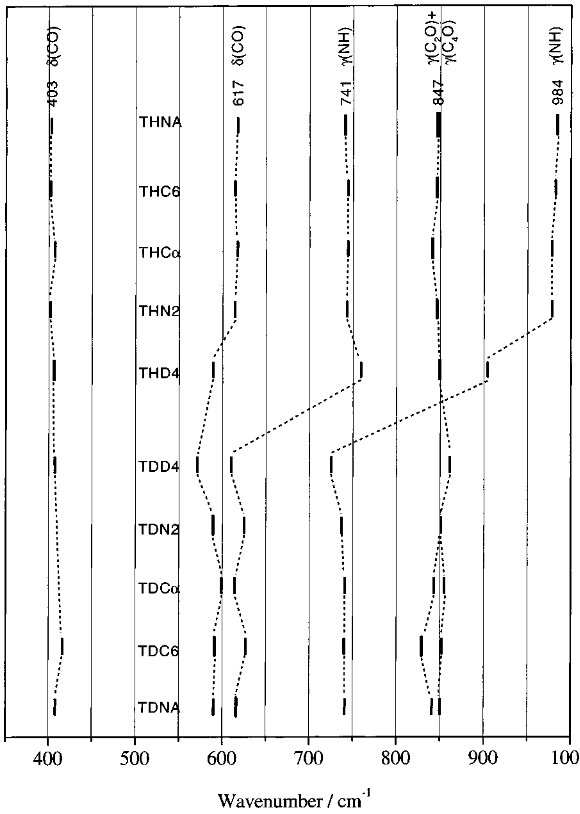

Figure 8. IR correlation diagram for the CO in-plane and out-of-plane bending bands and NH out-of-plane bending bands as per Figure 7. No bands are observed where no solid line is drawn for an isotopic derivative.

Weak IR bands at 403 and 617 cm^{-1} are tentatively assigned as the out-of-phase and in-phase CO bends, respectively, but are mixed with other internal coordinates. The two CO bending vibrations are weakly coupled through the N_3H between them. The CO bending frequencies in the N-deuterated species are significantly lower than those in the N-protonated species, which indicates some involvement of the ND wagging vibrations (Figure 8, vide infra). Also, the CO bending frequencies in THD4 and TDD4 are lower than in all the other isotopomers, consistent with a contribution from the in-plane C−CH_3 bend (vide infra).

Assignment of the 617 cm^{-1} band to the mixed, in-phase CO bend is supported by a structural argument. Geometrically, the in-phase bending is most likely accompanied by the C−CH_3 bending motion in the opposite direction; for the out-of-phase CO bending assigned at 403 cm^{-1} (IR) and 399 cm^{-1} (Raman), the involvement of the C−CH_3 bending is not required. The assignment of these two bands is identical with that of Florián and Hrouda[20] and Susi and Ard[18] (Table 1). In Mathlouthi et al.'s assignment,[19] the 617 cm^{-1} is assigned to a CO bend, but the other CO bend is considered at 432 cm^{-1} and the 403 cm^{-1} band is not observed. Differentiation of the 403 cm^{-1} in-plane CO bending band and the 432 cm^{-1} ring torsion band (vide infra) is only possible with a polarization study of single-crystal thymine, which was not attempted here.

NH Wags. Two NH wags are expected below 1000 cm^{-1}. The relevant IR bands are plotted in Figure 8. An NH wag is identified at 984 cm^{-1} in the IR spectra because no band is observed near this frequency in the spectra of the N-deuterated species. This NH wag is found at a much lower frequency in THD4 because of coupling between this vibration and the out-of-plane CD_3 deformation, as discussed above. For the N-deuterated species, the NH wag is shifted to even lower frequency, ca. 740 cm^{-1} in the IR and ca. 730 cm^{-1} in the Raman. This band also downshifts upon ^{15}N substitution, from 984 to 978 cm^{-1} for THN2 and from 741 to 737 cm^{-1} for TDN2, respectively.

The second NH wag is assigned to a weak band at 741 cm^{-1} in the IR. This NH wag also shifts upon N-deuteration to lower frequency, about 620 cm^{-1}, as shown in Figure 8. This band in THNA is also assigned to an accidentally degenerate ring stretch (vide infra) because only one band is observed in THNA, while two bands are seen in the other N-protonated species. This weak band is absent from the Raman spectra of many of the isotopomers.

Past assignments of the NH wags have been inconsistent. In Ar matrix-isolated thymine, the NH wags are assigned[22−24] at 662 and 545 cm^{-1}. In the polycrystalline state, these vibrations are expected to be at much different frequencies, due to hydrogen bonding as discussed previously. Mathlouthi et al.,[19] assigned only a broad band at 845 cm^{-1} to the NH wags, inconsistent with the spectra of the N-deuterated isotopomers reported here; this band is best assigned to the CO wags, as discussed above. In a recent study of polycrystalline thymine,[20] the NH wags are assigned at 761 and 560 cm^{-1}, also inconsistent with our spectra of the N-deuterated species. These two bands are best assigned to ring vibrations, on the basis of the consistent band frequencies and intensities observed for all the isotopomers (vide infra).

C_6H Bend. The C_6H in-plane bending mode is expected between 1200 and 1400 cm^{-1}. In the discussion above, the 1366 cm^{-1} IR band is assigned to the CH_3 symmetric deformation, while its Raman counterpart is observed at 1367 cm^{-1}, the second most intense band in the Raman spectrum (curves B and D in Figures 1−4). This Raman band seems too strong to be due to the CH_3 symmetric deformation alone. Therefore, we assign this band to both the CH_3 symmetric deformation and the C_6H in-plane bend. The C_6H in-plane bending component of the 1367 cm^{-1} band is shifted to 1135 cm^{-1} in THD4 and 1133 cm^{-1} in TDD4 upon C_6-deuteration (Figure 9). In previous studies,[18−20] this in-plane mode has been assigned to bands at 1246, 1366 and 1406 cm^{-1} and is consistently considered as having strong mixing with a ring vibration. Ab initio calculations[20] have also indicated that the C_6H in-plane mode is heavily mixed in several bands, with its greatest contribution to the 1406 cm^{-1} band. This is inconsistent with our isotopic spectra, which show the 1406 cm^{-1} band in all spectra of the N-protonated species, but none of the N-deuterated species. The 1406 cm^{-1} band is best assigned to an NH in-plane bend, as discussed above.

The C_6H wag is assigned at 935 cm^{-1} in this study. In contrast to the in-plane mode of the C_6-H bond, the out-of-plane mode shows a clear isotopic shift upon ^{13}C substitution at C_6 in THC6, which supports this assignment (Table 1). In previous work,[19,20] this mode is assigned at 1048 and 1152 cm^{-1}. These two modes are assigned here to the methyl rock and wag, respectively, because of the shift observed upon ^{13}C substitution at the methyl C and lack of shift upon ^{13}C substitution at C_6.

$C-CH_3$ Vibrations. Three vibrations are associated with the C_5-C_{11} bond: the stretch, bend, and wag. The $C-CH_3$ stretch can be assigned to an IR band at 1215 cm^{-1} (Figure 9). This band shifts to 1207 cm^{-1} in THCα and is also at a lower frequency in THC6 and THN2, indicating that it mixes with a ring stretching vibration, probably at ca. 1250 cm^{-1}. Methyl deuteration causes a significant shift of this band to 1156 cm^{-1} in THD4 and 1175 cm^{-1} in TDD4, as expected. Previously, the $C-CH_3$ stretch has been assigned to bands at[19] 760 cm^{-1}, at[18] 1203 cm^{-1}, and at[20] 1260 cm^{-1}, all of which are inconsistent with the shifts upon isotopic substitution observed here.

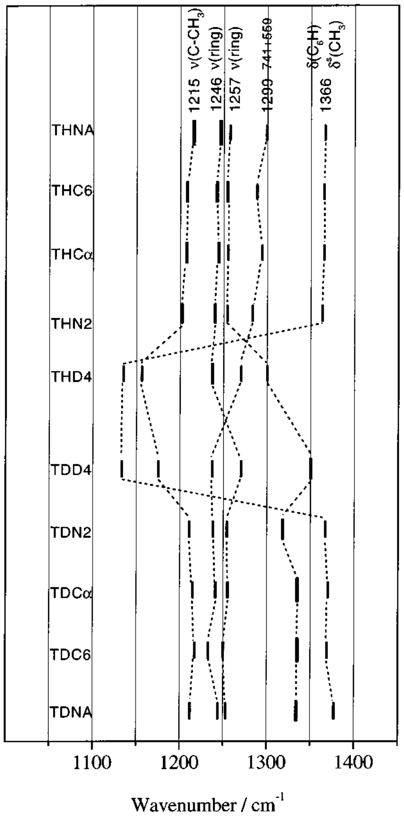

Figure 9. IR correlation diagram for the ring stretching, $C-CH_3$ stretching, and C_6H in-plane bending bands as per Figure 7.

A pair of bands are found at 284 and 321 cm^{-1} in the IR spectra, and at 287 and 319 cm^{-1} in the Raman spectra. Their association with the $C-CH_3$ bend and wag is clearly indicated by shifts to 255 and 302 cm^{-1}, respectively, when the methyl group is deuterated in THD4 and TDD4. While these bands can be definitively considered to originate from the $C-CH_3$ bend and wag, it is not obvious which vibration should be assigned at the higher frequency.[18,20] We tentatively assign the 321 cm^{-1} band to the $C-CH_3$ wag and the 284 cm^{-1} band to the $C-CH_3$ in-plane bend, on the basis of previous ab initio calculations.[20,24,26]

Ring Stretches. The five other ring stretching bands, in addition to the previously assigned C=C stretch, are assigned to bands at 1482, 1257, 1246, 814, and 741 cm^{-1}. Overall slightly lower frequencies for the 1482 cm^{-1} band in the N-deuterated species than in the N-protonated species indicate a small participation of the NH in-plane bending vibrations in this ring stretch, as discussed above.

The 1257 cm^{-1} ring band undergoes large shifts upon deuteration at all sites in the molecule (Figure 9). The upshifts upon deuteration at C_6 and C_α (THD4) indicate mixing with the CH_3 symmetric deformation and/or C_6H bend. A further upshift upon additional N-deuteration (TDD4) indicates mixing with the higher-frequency NH bends, which is removed upon N-deuteration.

In this region (Figure 9), a strong IR band is seen at 1246 cm^{-1} and a weak IR band is observed at 1299 cm^{-1}. The 1246 cm^{-1} band exhibits similar shifts upon isotopic substitution as the ring stretch at 1482 cm^{-1}, but shows a slightly larger frequency downshift for the THC6 and TDC6 isotopomers. The 1246 cm^{-1} band is therefore assigned as a ring stretch. The 1299 cm^{-1} weak band is assigned to a combination vibration of 741 + 559. At first glance, the correlation of the 1257 and 1299 cm^{-1} modes with isotopic substitution appears unusual. The band at 1299 cm^{-1} in THD4 and 1268 cm^{-1} in TDD4 must be correlated with the 1257 and 1246 cm^{-1} ring stretches, respectively, because of the strong intensity of these features in the Raman spectrum (Figure 5). In fact, this unusual pattern is also consistent with shifts in the 559 and 741 cm^{-1} fundamental bands that comprise the 1299 cm^{-1} combination band as a function of isotopic substitution.

The last two ring stretching bands at 814 and 741 cm^{-1} are expected to contain a significant contribution from ring deformations. These bands are shifted to lower frequency in the N-deuterated species, presumably due to the coupling with the nearby ND bend at about 900 cm^{-1}. All of these assignments are consistent with past assignments.[18,20]

Ring Bends and Torsions. Three bands are expected for each of the ring bending modes and torsion modes. The bending modes are assigned at the 475, 559, and 1030 cm^{-1} bands, and the ring torsions are assigned at the 209, 432, and 761 cm^{-1} bands. The 1030 cm^{-1} band is shifted to slightly higher frequency upon N-deuteration. This frequency shift may be caused by mixing with the ND bend near 900 cm^{-1} in this mode, pushing this band to a higher frequency. The assignment of the 475 cm^{-1} band to a ring bend and the 432 cm^{-1} band to a ring torsion is opposite to that reported previously,[20] but is more consistent with a polarization study[30] of 1-methyluracil that indicates that the higher wavenumber band may be more likely the in-plane bend.

The 209 cm^{-1} band has been previously assigned to the methyl torsion.[31] However, this assignment cannot be correct because of the lack of observed frequency shift upon methyl deuteration in THD4 and TDD4; the methyl torsion should be affected by deuteration. On the other hand, the IR and Raman frequencies for this band in the N-protonated species are not coincident. Specifically, the band frequencies are all near 196 cm^{-1} in both IR and Raman spectra of the N-deuterated isotopomers, but for the N-protonated isotopomers, the IR frequencies are near 209 cm^{-1} while the Raman frequencies stay at about 196 cm^{-1}. It is not obvious whether the 209 cm^{-1} IR band has the same origin as the 196 cm^{-1} Raman band, although they are tentatively treated in this way here (Table 1). These bands are all too high for lattice vibrations, and a previous polarization study of 1-methyluracil and 1-methylthymine confirms that this band originates from an out-of-plane vibration, which was considered[30,32] as a ring torsion.

CH_3 Torsion and Lattice Vibrations. The thymine crystal is monoclinic with the space group $P2_1/c$.[33] Adjacent molecules in the crystal form four hydrogen bonds to neighbors. Two hydrogen bonds are formed between the N_1H and C_2O in one molecule and the C_2O and N_3H in a second molecule. Two more hydrogen bonds are formed between the N_3H and C_2O in the first molecule and the C_2O and N_1H in a third molecule. Thus, the two pairs of intermolecular hydrogen bonds are expected to yield four bands in the low-wavenumber region.

In the spectra reported in this paper, low-frequency modes in natural abundance thymine are observed at 171, 99 and 75 cm^{-1} in the IR, and 172, 153, 115, 91, and 75 cm^{-1} in the Raman. Similar low-wavenumber bands have been observed in single crystals of 1-methyluracil and 1-methylthymine and have been assigned to symmetric and antisymmetric intermolecular stretching vibrations arising from hydrogen bonds in the crystal.[32,33] A normal coordinate analysis of the low-frequency bands of 1-methyluracil constrained by ultraviolet resonance Raman intensities supports these assignments of the hydrogen-bond vibrations.[31] Thus, the low-frequency modes at 75 and 99 cm^{-1} in THNA are assigned to the antisymmetric hydrogen-bond stretches. The 115 and 91 cm^{-1} bands, observed only in the Raman spectrum, are assigned to the symmetric hydrogen-bond stretches. We then assign the weak 171 cm^{-1} IR band to the 75 + 99 cm^{-1} combination vibration. The band at 153 cm^{-1}, only observed in the Raman spectrum, is at a slightly lower frequency in the N-deuterated derivatives and is further shifted to lower frequencies in THD4 and TDD4. We tentatively assign this band to the CH_3 torsion, which may be coupled with the ring torsion mode at 209 cm^{-1}.

Conclusions

In this paper, the IR and Raman spectra of 2-thiothymine and 10 isotopic derivatives of thymine have been presented. The IR and Raman spectra of the majority of these isotopomers have not been reported previously and represent a solid experimental foundation for describing the normal modes in thymine. The vibrational spectrum of polycrystalline thymine has been assigned with the use of these derivatives. Many of the inconsistencies in previous vibrational assignments of thymine have been clarified by the spectra of the isotopic derivatives presented in this study. Many of the vibrations are assigned with confidence, and qualitative estimates of the nature of coupling between the various internal coordinates making up the normal modes have been deduced.

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Supporting Information Available: Table 2 listing the observed FT-IR and FT-Raman frequencies of 2-thiothymine (1 page). Ordering information is given on any current masthead page.

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