Solution structures of purine base analogues 9-deazaguanine and 9-deazahypoxanthine

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Deaza analogues of nucleobases are potential drugs against infectious diseases caused by parasites. A caveat is that apart from binding their target parasite enzymes, they also bind and inhibit enzymes of the host. In order to design derivatives of deaza analogues which specifically bind target enzymes, knowledge of their molecular structure, protonation state, and predominant tautomers at physiological conditions is essential. We have employed resonance Raman spectroscopy at an excitation wavelength of 260 nm, to decipher solution structure of 9-deazaguanine (9DAG) and 9-deazahypoxanthine (9DAH). These are analogues of guanine and hypoxanthine, respectively, and have been exploited to study static complexes of nucleobase binding enzymes. Such enzymes are known to perturb pK_a of their ligands, and thus, we also determined solution structures of these analogues at two, acidic and alkaline, pH. Structure of each possible protonation state and tautomer was computed using density functional theoretical calculations. Species at various pHs were identified based on isotopic shifts in experimental wavenumbers and by comparing these shifts with corresponding computed isotopic shifts. Our results show that at physiological pH, N1 of pyrimidine ring in 9DAG and 9DAH bears a proton. At lower pH, N3 is place of protonation, and at higher pH, deprotonation occurs at N1 position. The proton at N7 of purine ring remains intact even at pH 12.5. We have further compared these results with naturally occurring nucleotides. Our results identify key vibrational modes which can report on hydrogen bonding interactions, protonation and deprotonation in purine rings upon binding to the active site of enzymes.

Keywords: purine base analogues; protonation state; ultraviolet resonance Raman spectroscopy; density functional theoretical calculation; Raman shift; deuterium labeling; wB97XD

Introduction

Nucleobase analogues and their corresponding nucleosides and nucleotides are extensively used in enzymology as inhibitors, drugs, and probes. Deaza purines in particular are used as analogues of natural purine substrates to make stable enzyme–substrate complexes (Wierzchowski, Bzowska, Stepniak, & Shugar, 2004; Wierzchowski, Medza, Sepiol, Szabelski, & Shugar, 1996; Wierzchowski, Stepniak, Bzowska, & Shugar, 2005; Wierzchowski, WielgusKutrowska, & Shugar, 1996). Two well-known enzymes with purine substrates are hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and purine nucleoside phosphorylase (PNP). These enzymes are important in several disease contexts, e.g. in gouty arthritis and cellular immunodeficiency (Wevers et al., 1999). In general, recognition of nucleobases by such enzymes occurs through hydrogen bonding contacts of the purines with amino acid residues (Figure 1) and stacking interactions.

9-Deazapurines are good substrate mimics since they bind to these enzymes without undergoing catalysis. 9-deazaguanine (9DAG) and 9-deazahypoxanthine (9DAH) are analogues of guanine (Gua) and hypoxanthine (Hx), respectively, in which the nitrogen of the imidazole ring (N9) is substituted by a carbon atom (Figure 2). This substitution alters the electron distribution of the ring and results in stable hydrogen at N7 position of purine. Purine analogues have been employed as bisubstrate inhibitors of PNP (Holý et al., 1996; Mikleušević et al., 2011; Wielgus-Kutrowska, Antosiewicz, Długosz, Holý, & Bzowska, 2007; Wielgus-Kutrowska & Bzowska, 2006), and thus, these inhibitors shed light on both active site structure and dynamics. PNP catalyzes the reversible phosphorylation of nucleosides (guanosine and adenosine) and results in the formation of nucleobase and phosphorylated sugar. Derivatives of 9DAG have been exploited as a multi-substrate analogue inhibitors of PNP (Hikishima, Hashimoto, Magnowska, Bzowska, & Yokomatsu, 2007; Kolossavary & Guida, 1999). Previously, 9-deaza inosine derivatives have been identified as good inhibitors of PNP (K_i = 1.8 × 10^{-17}) (Stein et al., 1987; Stoeckler et al., 1986).

In another enzyme from the purine salvage pathway, HGPRT, co-crystal structures of the enzyme with 9-deazapurines were valuable in showing the interaction of the substrate with the enzyme. Crystal structure of 9DAG...
Toxoplasma gondii HGPRT not only suggested the distortion of this nucleobase toward the transition state but also indicated that the reaction proceeds through SN2 mechanism (Héroux, White, Ross, Kuzin, & Borhani, 2000). Tritrichomonas foetus HGPRT co-crystallized with 9DAG showed the dynamics of the catalytic loop which sequesters the transition state during the reaction. Inhibition of 9DAG led to the irreversible closure of this loop (Munagala, Basus, & Wang, 2001).

In this work, we have determined the solution structure and vibrational spectra of 9DAG and 9DAH. Since purines are well-known to populate different tautomeric and protonation states, it is important to reliably determine the solution structures of these molecules. We compare their structure with corresponding natural nucleobases, Gua and Hx, respectively. These will aid mechanistic understanding of inhibitor activity and improvement of inhibitor design.

Nucleobases in Immucillin HP (ImmHP) and Immucillin GP (ImmGP) (Figure 2) are 9DAH and 9DAG, respectively. Although pK_a of 9DAG and 9DAH are not known, three pK_a values of ImmH and ImmHP have been determined by Schramm and co-workers, viz. 1.4, 6.9, and 10 using solid and solution NMR chemical shifts. These represent deprotonation at N3, ribose N, and N1 position, respectively (Sauve et al., 2003). In previous work, we did not find the presence of N3 tautomer in 6-oxopurines at physiological pH (Gogia, Jain, & Puranik, 2009; Gogia & Puranik, 2014). Computational

Figure 1. 9DAG bound at the active site of Toxoplasma gondii HGPRT. The purine analogue is bound through a hydrogen bonding network which is similar to the one observed with natural substrates. This depicts the utility of such analogues in probing the active site at a molecular level and providing a rationale for the design of targeted inhibitors.

Figure 2. Structure of analogues of purine bases and nucleotides. Panel I shows proposed ribo-oxocarbenium ion (a) transition state of HGPRT catalyzed reaction and transition state analogues, ImmGP (b) and ImmHP (c). Panel II shows natural nucleobases guanine (a) and hypoxanthine (b), their corresponding analogues 9-deazaguanine and 9-deazahypoxanthine in neutral (c) and (d), acidic (e and f), and alkaline (g) and (h) pHs.
data have also suggested that the N3 protonated tautomer is a relatively high energy structure among the possible single protonation state (Hernández, Orozco, & Luque, 1996). It was also shown that although in the gas phase N9 deprotonated tautomer is more stable than that of deprotonated at N1, by 9.3 kJ mol\(^{-1}\), both the tautomers had the same energy when placed in a dielectric continuum (Giese & McNaughton, 2002). We have previously shown that even when the chemical structures of nucleobases and their analogues are very similar, they have different characteristic vibrational spectral patterns in resonance Raman (RR) spectra (Gogia et al., 2009; Jayanth, Ramachandran, & Puranik, 2009). The RR spectra of nucleobases are highly sensitive to pH reflecting the effect of protonation and deprotonation on the structure. Further, the use of ultraviolet excitation wavelengths in resonance with the allowed \(\pi-\pi^*\) state provides a large enhancement in the Raman cross sections of these molecules. These properties make vibrational spectroscopy of nucleobases a very useful, label-free technique to study nucleic acid binding enzymes.

Absorption spectra of 9DAG and 9DAH at pH 1, pH 7, and pH 10 are shown in Figure 3. Ultraviolet resonance Raman (UVRR) spectra of 9DAG and 9DAH were acquired at neutral, acidic, and alkaline pH in the wavenumber range 1200 to 1800 cm\(^{-1}\). In-plane base vibrations such as C=O, C=N, C=C, and –NH\(_2\) bending vibrations are located in this region. Various bands associated with different vibrational modes were assigned using density functional theory (DFT) calculations. We find that unlike the localized mode of carbonyl (C=O), purine ring modes are highly coupled and delocalized over the entire ring. Both molecules exist in a single tautomeric state in solution. At pH 7, only one protonation state, neutral form, dominates the observed spectra.

**Experimental and theoretical methods**

**Sample preparation for absorption and UVRR spectroscopy**

Initial samples of 9DAG and 9DAH were the gift from Dr Vern Schramm. Subsequently, these were purchased from Sigma-Aldrich and used without any further purification. 25 mM stock solution of these nucleobases was prepared in 0.2 N NaOH. Concentration of nucleobases in all UVRR experiments was 500 \(\mu\)M and was prepared in 20 mM Tris-HCl. pH of the sample was adjusted by dissolving appropriate amount of 13 N HCl and 10 N NaOH for the acidic and basic pH, respectively. To prepare the samples in D\(_2\)O, NaOD, and DCl was used. To ensure complete exchange of labile protons, the samples were kept overnight in deuterated buffers. For absorption spectra, nucleobase solution of 50 \(\mu\)M was prepared in Tris-HCl buffer of suitable pH for neutral, acidic, and alkaline solutions.

**UVRR spectroscopy**

The UVRR spectra were measured using 260 nm excitation wavelength generated by tunable Ti-Sapphire laser (Indigo, Coherent Inc.). A detailed description is provided previously (Jayanth et al., 2009). The average power used at the sample was ~0.6 mW. Calibration was done by recording spectra of solvents, dimethylformamide, cyclohexane, indene, acetonitrile, trichloroethylene, and isopropanol, with the known band positions.

**Data acquisition and analysis**

Each spectrum is the average of three samples, each of which was acquired over ~15 min. The absence of photodamage was confirmed by comparing the first and
last spectrum acquired. SynerJY (Jobin-Yvon) was used for data acquisition and analysis. Raman bands were fitted using Lorentzian line-shape function to obtain the wavenumber of the spectrum.

Computational methods
Quantum chemical calculations were performed on 9DAG and 9DAH to obtain energy minimized structure. Density functional theoretical formalism was employed using wB97XD (Chai & Head-Gordon, 2008) with a 6-31G (d, p) basis set, as implemented in Gaussian 09 (Frisch et al., 2009). Raman wavenumber calculations were done on the optimized structure. To calculate the structure of the charged molecule, a proton was added or removed from the neutral molecule using GaussView 5.0 (Dennington, Keith, & Millam, 2009). Following which the structure was optimized. Potential energy distribution calculations were performed using the software VEDA 4.0 (Jamroz, 2004) (vibrational energy distribution analysis). Experimentally observed shifts due to isotopic labeling were computed by replacing the mass at the labile hydrogens with the mass of deuterium in the molecules. Normal mode description was inferred by visualizing the vibrational modes in the software Chemcraft 1.6 (http://www.chemcraftprog.com). Diagrams depicting normal mode contributions were generated by Chemcraft. In molecules where more than one tautomer is possible, all the possible tautomers were optimized and the Raman wavenumber calculations were done. These were further compared with experimentally observed Raman wavenumbers.

Results and discussion
UVRR spectra of 9DAG at pH values 1.5, 7, and 12.5 were recorded. These spectra were compared with the computed spectra of 9DAG in neutral, cationic form protonated at N3, and anionic form deprotonated either at N1 or N7 (Figure S1). The spectrum at pH 7 is assigned to the neutral species with hydrogens on N1 and N7 based on its comparison with the computed spectrum of the same species (Figure 4 and Table 1). To decipher normal mode compositions of the observed bands, spectra were also obtained by replacing the amine and imine hydrogens with deuterium. For this, the samples were dissolved in D2O solution leading to the replacement of hydrogens with deuterium at position N1, N2, and N7 (Figure 4). Assignments of experimental bands were made based on the comparison of observed isotopic shift with the corresponding computed shift. Normal mode vectors obtained from computational calculations are shown in Figure S2. Raman spectrum consists of the entire set of in-plane vibrations of the nucleobases and vibrations of the non-planar –NH2 moiety. This moiety is non-planar due to the pyramidalization of exocyclic –NH2 group of Gua which happens due to partial SP3 hybridization. The –NH2 moiety experiences repulsion from the proton of N1–H group of pyrimidine ring.

Neutral, protonated, and deprotonated forms of 9-deazaguanine
Carbonyl stretch
The band at 1684 cm⁻¹ in the UVRR spectrum of 9DAG at pH 7.0 is attributed to carbonyl stretch (Table 1). This shows a downshift of 21 cm⁻¹ upon deuteration.
suggesting the coupling of carbonyl stretch with N1–H of purine ring. In 9DAG⁺, this band shows a huge downshift of 81 cm⁻¹ indicating a significant loss of the double-bond character of the C6–O6 bond (Table 2). This suggests that at high pH, the proton at N1 is lost and the electron density in the pyrimidine ring is increased. Furthermore, the species at pH 12.5 does not show any isotopic shift in the position of the carbonyl bond, indicating that there is no contribution from an N1–H coordinate in this mode and corroborating the interpretation that the proton is lost from N1 position of the pyrimidine ring. In 9DAGH⁺, carbonyl mode is at 1715 cm⁻¹ (Table 3). DFT calculations show an upshift of 62 cm⁻¹ from the neutral form which results from the greater double-bond character of carbonyl following protonation at N3 position. This sensitivity of carbonyl makes it a good marker of the protonation state. At the active sites of enzymes, this mode serves as a marker of hydrogen bonding interaction between the enzyme and the nucleobase.

### Pyrimidine ring distortion

The band at 1658 cm⁻¹ in the UVRR spectrum of neutral 9DAG represents the stretching vibrations (str) of C2N3 and C2N2 along with the scissoring vibrations (sci) of −NH₂. A large deuterium isotopic shift is observed (62 cm⁻¹). DFT calculations show that the downshift is due to decoupling of −NH₂ sciss from this mode upon deuteration. A small, 2 cm⁻¹ isotopic downshift observed in deprotonated 9DAG (1589 cm⁻¹ in H₂O and 1587 cm⁻¹ in D₂O) suggests decoupling of −NH₂ upon deuteration in deprotonated form. Opposite effects are observed on protonation. This band upshifts in 9DAG⁺ suggesting that the double-bond character is enhanced upon protonation. Sensitivity of this mode in 9DAG⁺ to deuterium labeling confirms the contribution of −NH₂ sci and N1–H. Thus, this mode can act as a reporter of hydrogen bonding interaction between protein–nucleobase at −NH₂ position in enzyme-bound forms of 9DAG.

### Table 1. Experimental resonance Raman shifts of neutral 9-deazaguanine in water and D₂O at pH 7.0 and computed (wB97XD/6-31G (d, p)) vibrational wavenumbers of neutral 9-deazaguanine with mode assignments².

| UVR in H₂O | DFT | Mode assignments | UVR in D₂O | DFT | Mode assignments |
|------------|-----|----------------|------------|-----|-----------------|
| 1682       | 1850 | Str C6O6 (69%)  | 1661       | 1843 | Str C6O6 (71%)  |
| 1658       | 1721 | Str C2N3 (50%) + C2N2 (−12%) + Sci N2H₂     | 1596       | 1693 | Str C2N3 (59%)  |
| 1608       | 1655 | Sci N₂H₃ (55%)  | 1391       | 1258 | Sci ND₂ (32%) + Be N1DC6 (−13%) |
| 1547       | 1624 | Str C4C5(42%)-C8C9 (−16%) + Str N3C4 (−11%) + Be N7H₁ + N1H + NH₂ | 1574       | 1616 | Str C4C5 (47%) + Str C8C9 (−28%) + Str N3C4 (−15%) |
| 1452       | 1595 | Str C4C5N7 (16%) + Be N3C4C5 (14%) + Sci NH₂ + Be C9H + C8H + N1H | 1432       | 1588 | Be N3C4C5 (18%) + Be C4C5N7 (17%) + Str C2N2 (10%) + Str N1C2N3 (10%) |
| 1532       | 1542 | Be N7C8H (15%) + Str C8C9 (32%) | 1535       | 1530 | Str C8C9 (32%) + Be C8H₂C9 (−15%) |
| 1477       | 1516 | Be N7HC₅ (22%) + Str C5N7 (−20%)-N7C8 (−13%) + Be N7C8C9 (−11%) + N1H | 1457       | 1485 | Str C5N7 (32%) + Be N7C8C9 (19%) + Str C4C5 (−11%) |
| 1418       | 1485 | Str N1C2 (−10%) + Str C4C5 (29%) + Be C4C5N7 (−11%) | 1412       | 1461 | Str N1C2 (31%) + Str C4C5 (−13%) + Str C2N2 (−15%) + Be N2D₂a |
| 1373       | 1406 | Str N3C4 (29%) + Str C5N7 (−21%) + Str N7C8 (−14%) + Be N1H | 1352       | 1388 | Str N3C4 (25%) + Str N7C8 (−22%) |
| 1343       | 1344 | Be N1HC₆ (29%) + Str C2N2 (10%) + Rock NH₂b | 1306       | 1329 | Be N7C8H (13%) + Str N1C6 (15%) + Be C9H₈ (13%) + Be N1H₆ (−12%) + Str C2N₂ (−12%) |
| 1230       | 1248 | Str N1C₆ (−17%) + Be N7HC₅ (23%) + Str N7C₅ (13%) | 1154       | 1184 | Str N1C₆ (21%) + Be N₂D₂ (18%) + Be N7D + Be C9H |
| 1177       | 1179 | Str N7C₈ (27%) + Str N1C₆ (−10%) + Be N2H₂b (14%) | 1147       | 1089 | Str C2N₂ (15%) + Sci N₂D₂ (−14%) + Be N₁C₆-O (−13%) + Be N₁D₂C (−10%) |
| 1147       | 1144 | Be N7C₈H (28%) + Be N2H₂b (−18%) + Str N7C₈ (24%) + Str N1C₆ (−9%) | 1336       | 1320 | Str N1C₆ (24%) + Str N7C₈ (18%) + Be C₈H₉C (−11%) |
| 1120       | 1114 | Be C₉H₈C (16%) + Be C₅N₇H (−15%) + Be C₅N₇C₈ (−13%) + Str N7C₈ (11%) + Str C₂C₈ (10%) | 1291       | 1193 | Be C₈H₉C (16%) + Be C₉H₈C (−14%) + Str C₅N₇ (−11%) + Be C₅N₇C₈ (10%) |
| 1096       | 1096 | Be C₉H₈C (27%) + Be N7C₈H (−17%) + Be C2N₂H₂ (−14%) + Str C₂C₈ (11%) | 1235       | 983  | Be C₂N₂D₂a (21%) + Be N₁D₂C (18%) + Str C₂N₃ (11%) + Be N7D + Be C₉H |

²Abbreviations: DFT, Density functional theory; Str, Stretch; Be, Bend; Py, Pyrimidine; Sci, Scissors; Pu, Purine.
Table 2. Experimental resonance Raman shifts of deprotonated 9-deazaguanine in water and D$_2$O at pH 12.5 and computed (wB97XD/6–31G (d, p)) vibrational wavenumbers of deprotonated 9-deazaguanine with mode assignments$^a$.

| UVRR in H$_2$O | DFT | Mode assignments | UVRR in D$_2$O | DFT | Mode assignments |
|----------------|-----|-----------------|----------------|-----|-----------------|
| 1602           | 1736| Str C6O6 (70%)  + Be C4C5N7 (10%) + Be NH2 (30%) | 1602           | 1735| Str C6O6 (71%)  + Be C4C5N7 (22%) |
| 1589           | 1624| Str C2N3 (18%)  + Be C4C5N7 (10%) + Be NH2 (30%) | 1587           | 1610| Str C2N3 (30%)  + Be C4C5N7 (22%) |
| 1540           | 1618| Be C4C5N7 (20%) + Be NH2 (35%)            | 1156           | 1175| Be C4C9H (10%)  + Be ND2 (23%) |
| 1551           | 1643| Str C8C9 (-14%) + Str C4C5 (34%) + Str N3C4 (-12%) | 1548           | 1638| Str C8C9 (-14%) + Str C4C5 (34%) + Str N3C4 (-16%) |
| 1502           | 1514| Str C2N3 (11%)  + Str C4C5 (14%) + Str N7C8 (-11%) + Be C4C5N7 (-11%) | 1498           | 1505| Str C2N3 (16%)  + Str C4C5 (10%) + Str C2N2 (-12%) + Be C4C5N7 (-15%) + Be N1C2N3 (-10%) |
| 1323           | 1548| Str C8C9 (30%)  + Str C2N3 (-10%) + Be C8HC9 (-20%) | 1320           | 1539| Str C8C9 (33%)  + Be C8HC9 (-23%) + Be C5N7C8 (12%) |
| 1397           | 1473| Str C4C5 (-12%) + Str C5N7 (22%) + Be N7HC8 (12%) + Be C8HC9 (13%) | 1358           | 1436| Str N1C2 (12%)  + Str N7C8 (19) + Str C5N7 (-16%) + Be C4C9H (10%) + Be N7HC8 (-10%) + Be N7C8C9 (-17%) |
| 1443           | 1362| Str N3C4 (28%)  + Str C5N7 (-18%)            | 1449           | 1366| Str N3C4 (-29%) + Str C5N7 (18) + Str N2C2 (10%) + Be C8HC9 (12%) |
| 1308           | 1312| Str N1C6 (24%)  + Str N7C8 (10%)            | 1296           | 1311| Str N1C6 (33%)  + Str N7C8 (23%) + Be C8HC9 (-14%) + Be ND2 (12%) |
| 1165           | 1244| Str N1C6 (22%)  + Be N7HC8 (16%) + Be C2N2H (10%) | 1138           | 947 | Be N7HC8 (-17%) + Be C2N2Ha (-22%) + Str N1C6 |
| 1226           | 1208| Str N7C8 (19%)  + Be N7HC8 (11%) + Be C2N2Ha (-11%) + Be C2N3C4 (-12%) + Be C5N7C8 (12%) | 1200           | 1169| Be ND2 (-18%) + Be C2N3C4 (-10%) + Be C5N7C8 (13%) |

$^a$Abbreviations: DFT, Density functional theory; Str, Stretch; Be, Bend; Py, Pyrimidine; Sci, Scissors; Pu, Purine.

Table 3. Experimental resonance Raman shifts of protonated 9-deazaguanine in water and D$_2$O at pH 1.5 and computed (wB97XD/6–31G (d, p)) vibrational wavenumbers of protonated 9-deazaguanine with mode assignments$^a$.

| UVRR in H$_2$O | DFT | Mode assignments | UVRR in D$_2$O | DFT | Mode assignments |
|----------------|-----|-----------------|----------------|-----|-----------------|
| 1715           | 1912| Str C6O6 (78%)  + Str C5C6 (-14%) | 1700           | 1906| Str C6O6 (-79%) + Str C5C6 (14%) |
| 1664           | 1752| Str C2N3 (-15%) + Str C2N2 (34%) + Be N3HC4 (-10%) + Be NH2 (11%) | 1625           | 1704| Str C2N3 (-20%) + Str C2N2 (38%) + Be N1C2N3 (-18%) |
| 1601           | 1696| Str C4C5 (-13%) + Str C2N3 (12%) + Be NH2 (20%) | 1584           | 1671| Str C4C5 (24%) + Str N1C2 (16%) + Str C2N3 (-14%) |
| 1549           | 1615| Str C4C5 (21%)  + Str N1C2 (-13%) + Str N3C4 (-13%) | 1552           | 1610| Str C4C5 (-17%) + Str N1C2 (-11%) + Str N3C4 (18%) |
| 1453           | 1591| Str C2N3 (-12%) + Be C4C5N7 (14%) + Be N7C8H (11%) | 1436           | 1564| Str C2N3 (-12%) + Str C8C9 (11%) + Str N1C2 (18%) + Be C8HC9 (-16%) |
| 1417           | 1510| Str C8C9 (29%)  + Str N7C8 (-15%) + Be N7C8H (21%) | 1415           | 1485| Str C8C9 (27%) + Str C5C6 (-11%) + Be C4C5N7 (-12%) + Be C8HC9 (-11%) |
| 1533           | 1546| Str C5N7 (26%)  + Be N7HC8 (22%) + Be N7C8C9 (18%) | 1481           | 1514| Str C5N7 (32%) + Be C8HC9 (26%) |
| 1377           | 1363| Str C2N2 (16%)  + Str C5N7 (-11%) + Be N3HC4 (20%) + Be N1HC2 (-13%) + Be N1HC8 (11%) | 1400           | 1416| Str N7C8 (27%) + Str N3C4 (-14%) + Be C4C9H (15%) |
| 1355           | 1406| Str N7C8 (-13%) + Be N1HC2 (35%) | 1300           | 1323| Str N7C8 (-17%) + Be ND2 (13%) |
| 1296           | 1248| Str N7C8 (-15%) + Be N7HC8 (-16%) + Be C5N7C8 (-14%) | 1246           | 1206| Be C8HC9 (10%) + Be C4C9H (18%) + Be C5N7C8 (15%) |
| 1228           | 1120| Str C8C9 (15%)  + Str N7C8 (12%) + Be C2N2Ha (-16%) | 1362           | 1179| Str C8C9 (12%) + Str N7C8 (13%) + Be N3HC4 (-16%) + Be N1C2 (-16%) + Be ND2 (-11%) |
| 1142           | 1116| Str N1C2 (-15%) + Be C8HC9 (30%) | 1166           | 1125| Str C2N2 (-10%) + Be C8HC9 (18%) + Be C4C9H (10%) + Be ND2 (27%) |

$^a$Abbreviations: DFT, Density functional theory; Str, Stretch; Be, Bend; Py, Pyrimidine; Sci, Scissors; Pu, Purine.
NH₂ scissoring mode
The band at 1608 cm⁻¹ in the spectrum of neutral 9DAG is attributed to –NH₂ sci. DFT calculations predict an isotope labeling induced downshift of 397 cm⁻¹. Correspondingly, we assigned the band with the largest isotope-induced shift at 1391 cm⁻¹ of 9DAG (~217 cm⁻¹ in D₂O) to this mode. In deprotonated 9DAG, this band appears at 1540 cm⁻¹ with a slightly different normal mode composition (Figure S3). There, the –NH₂ sci is coupled to C4C5N7 bending vibration (bend). In 9DAGH⁺, the band appears at 1601 cm⁻¹ in water, upon deuteration decouples from –NH₂ and appears at 1584 cm⁻¹.

C–N stretch coupled to C–C stretch
The band at 1547 cm⁻¹ in the UVRR spectrum of 9DAG at pH 7 is ascribed to the str of C4–C5 and C8–C9 coupled to N3–C4. Minor contribution from N7H, N1H str, and NH₂ bend is also predicted by DFT. While calculations predict a downshift in this band upon deuteration, a large upshift of 27 cm⁻¹ is observed. This upshift implies decoupling of N1H and –NH₂ bend on deuteration. The disparity shows that DFT calculations underrate the contributions of bend to this mode. The band shows only a slight upshift in protonated 9DAG indicating that protonation at N3 of pyrimidine ring results in a more localized mode.

Triene stretch
The band at 1452 cm⁻¹ is the most intense band in the spectrum of 9DAG in water at pH 7 and is attributed to the in-plane C4–C5, C5–N7 str and N3–C4–C5 bend. DFT calculations predict a downshift of 7 cm⁻¹ against an experimental observation of a downshift of 20 cm⁻¹ indicating contribution from N1–H str and –NH₂ bend. In 9DAG⁻, this mode appears at a higher shift at 1551 cm⁻¹. The upshift is due to the reorganization of the normal mode composition on deprotonation as predicted by DFT calculations. In 9DAGH⁺, this band appears at 1453 cm⁻¹. The mode is highly delocalized in D₂O and includes N1–C2 str leading to an isotopic downshift of 17 cm⁻¹.

C8–H bending mode
The band at 1532 cm⁻¹ is attributed to C8–C9 str and N7C8H bend. While a downshift is predicted by DFT calculations upon deuteration, a small upshift of 3 cm⁻¹ is observed. The isotope-induced upshift implies contribution of N1–H bend. The interpretation is further corroborated by the sensitivity of this band to deprotonation at N1. In 9DAG⁻ the band is observed at 1323 cm⁻¹. Protonation also leads to a downshift, so that in 9DAGH⁺, this band is observed at 1417 cm⁻¹.

Pyrrole ring vibrations
The band at 1477 cm⁻¹ in the spectrum of neutral 9DAG is attributed to pyrrole ring vibration coupled to C1–H bend. Deuteration causes a downshift of 20 cm⁻¹ while DFT calculations predict a downshift of 31 cm⁻¹. This band in neutral 9DAG can serve as a marker for hydrogen bonding at N7 position of purine with proteins. The observed shift upon H→D isotopic labeling increases from neutral to deprotonated and further to protonated 9DAG species indicating that the normal mode composition is reorganized with change in protonation state.

Purine ring mode
In 9DAG at pH 7, the band at 1373 cm⁻¹ is assigned to the purine ring mode. The detail composition of the normal mode is given in Table 1. This band downshifts upon deuteration as correctly predicted by DFT calculations. In 9DAG⁻, the deprotonation of N1 leads to the upshift of 70 cm⁻¹ (underestimated at 40 cm⁻¹ by DFT) in this mode. In 9DAGH⁺, the observed wavenumber is upshifted as a result of the strengthening of C–C and C–N bond upon protonation. The disparity between the shifts predicted by DFT calculations and the observed shifts may be due to the highly delocalized nature of the normal mode composition which extends over the purine ring. DFT calculations do not reproduce these shifts adequately.

Pyrimidine ring distortion
The band at 1417 cm⁻¹ in the UVRR spectrum of neutral 9DAG is ascribed to N1–C2 str and C4–C5–N7 bend. This band appears as a shoulder on the most intense band at 1452 cm⁻¹. Upon deuteration, the band downshifts to 1412 cm⁻¹. DFT calculations also predict the downshift of 24 cm⁻¹. In 9DAG⁻ and 9DAGH⁺, the band is not observed in the UVRR spectrum since it is expected to have downshifted below the range of observation in these experiments. In general, normal modes in the region from 1200 to 1450 cm⁻¹ wavenumber region have strong contribution from N–H bend and –NH₂ rocking vibrations.

Neutral, protonated, and deprotonated forms of 9DAH
9DAH is an analogue of Hx. While Hx is known to exist as a mixture of two tautomers, one with H7 and the other with H9 at pH 7, in 9DAH, the presence of carbon in place of nitrogen at 9th position results in the occurrence of only N7H at pH 7. UVRR spectra of
9DAH at pH 1.5, 7, and 10.5 in H₂O and D₂O are shown in Figure 5. These spectra were compared with the computed spectra of 9DAH in neutral, cationic form protonated at N3, and anionic form deprotonated either at N1 or N7 (Figure S4). Normal mode diagrams are shown in Figure S5. We compare the UVRR spectrum of 9DAH with that of IMP (Gogia et al., 2009). IMP exists in only one form where the sugar and phosphate at N9 position lead to the base corresponding to the N9H tautomer of Hx. Spectrum of 9DAH is quite distinct from that of Hx and IMP (Gogia et al., 2009). We find that 9DAH undergoes deprotonation at a higher pH. The site of deprotonation is N1 of the pyrimidine ring.

**Carbonyl stretch**

In contrast to infrared vibrational spectra, the carbonyl mode is usually weak in Raman spectra. It is observed at 1681 cm⁻¹ in 9DAH (Table 4). A downshift in the band upon deuteration in computed and experimental wavenumbers suggests a weak contribution of N1–H to this mode. Isotope-induced downshift in UVRR spectrum of 9DAH is 15 cm⁻¹ close to that in IMP (17 cm⁻¹) indicating similar normal mode compositions. Since the carbonyl bond is often the part of the nucleobase involved in intermolecular interactions with proteins and other nucleobases, these shifts indicate that the carbonyl moiety of the analogue can very well mimic Hx in this aspect. At pH 12.5, in 9DAH⁻ (Figure 5 and Table 5), deprotonation occurs at N1 position and the same is reflected in the form of downshift in carbonyl mode in both computed (1732 cm⁻¹) and experimental (1664 cm⁻¹) wavenumbers. In 9DAH⁺, no shift in this band upon isotope labeling indicates the absence of exchangeable proton at N1 position. In 9DAHH⁺ (Figure 5 and Table 6), the band appears at 1730 cm⁻¹ to show an upshift as compare to the wavenumber of neutral 9DAH. The absence of charge separation at C6=O position results in greater double-bond character in carbonyl bond which in turn cause an upshift in this band. Similar trend is also observed in IMP. This band can serve as a marker of hydrogen bonding interaction at N1–H and C6=O positions of purine ring.

**C2–N3 stretch coupled to bending vibrations**

The band at 1602 cm⁻¹ in the neutral 9DAH is mostly attributed to C2–N3 str coupled to N1–H, C2–H and N7–H bends. Computed and experimental wavenumbers show downshift upon deuterium labeling (H → D) which corroborates the involvement of labile protons. In 9DAH⁻, this band downshifts to 1598 cm⁻¹. A further downshift to 1595 cm⁻¹ upon deuterium isotopic labeling in 9DAH⁻ suggests a weak involvement of N7–H bend. In protonated 9DAH at pH 1.5, this band upshifts to 1653 cm⁻¹ which is also corroborated by the computed wavenumber where an upshift of 35 cm⁻¹ is observed as compared to neutral form.

**C–N stretch coupled to C–C stretch**

The band at 1523 cm⁻¹ in the UVRR spectrum of 9DAH arises from N3–C4 str of purine ring. At neutral pH, N3 remains deprotonated and thus any downshift in this band reflects the overall distortion in the purine ring moiety upon deuteration. In 9DAH⁻ also, N3 remains deprotonated and a slight downshift in the UVRR spectrum upon deuteration suggests ring distortion. At low pH, 9DAH acquires a proton at N3 and shows a huge downshift of 34 cm⁻¹ (29 cm⁻¹ computed wavenumber).
The band is an intense band in neutral and deprotonated forms and loses intensity in the spectrum of the protonated form. Thus, a loss in intensity of this band can be an indicator of perturbation in the protonation state.

**Pyrrrole ring mode**

The band at 1424 cm$^{-1}$ in the spectrum of neutral 9DAH is attributed to pyrrrole ring mode comprised of C4=C5 str coupled to the C5–N7–C8 and N7–C8–C9 bends. This is the most intense band in the spectrum of neutral 9DAH in water and downshifts to 91 cm$^{-1}$ upon deuteration. In 9DAH$^+$ at pH 12.5, this band appears at 1424 cm$^{-1}$ and is an intense band. The band at 1421 cm$^{-1}$ in the spectrum of 9DAH$^+$ is attributed to this mode with an additional contribution from N1HC2 bend. H→D substitution results in minor downshifts in all protonation states indicating that there is little contribution from the N–H str and bends into the ring mode.

**Pyrimidine ring mode**

The ring mode is intense as expected, at 1388 cm$^{-1}$ in the spectrum of neutral 9DAH. Vibrations contributing to this mode are C2HN3 bend, N1HC6 bend, and C2–N3, N3–C4 str. This band loses intensity and shifts down by 8 cm$^{-1}$ upon deuteration. Furthermore, in 9DAH$^+$ at pH 12.5, a meagerly intense band is present at 1368 cm$^{-1}$ which shows only a minor downshift of 1 cm$^{-1}$ upon deuterium isotopic labeling DFT predicts a downshift of 3 cm$^{-1}$. Similar downshifts upon H→D labeling in observed and computed spectra further corroborates that the site of deprotonation at pH 12.5 is N1 of pyrimidine ring. In 9DAH$^+$, this band appears at 1371 cm$^{-1}$. Calculations predict a downshift of 270 cm$^{-1}$ upon H→D labeling in 9DAH$^+$. A large reorganization of the normal mode composition is predicted by DFT calculations upon deuteration. The corresponding observed band in protonated 9DAH in H$_2$O is observed at 1371 cm$^{-1}$ which downshifts to a wavenumber region below the range of examination in these experiments. We predict that this band can act as a marker of hydrogen bonding interactions at N1H position of the purine ring when bound to the active site of enzyme. Changes in the intensity of this band from neutral to protonated and to deprotonated can report on the protonation state of this ligand at the active site of an enzyme.
Purine ring mode

A prominent band observed at 1346 cm\(^{-1}\) in the UVRR spectrum of neutral 9DAH is assigned to purine ring distortion mode. This band is comprised of C2HN3 and N7HC8 bends followed by N3–C4, C5–N7, and N7–C8 str. This band shows a downshift of 44 cm\(^{-1}\) upon deuteriation that is underestimated at 29 cm\(^{-1}\) by calculations. In 9DAH\(^+\), this band appears at 1341 cm\(^{-1}\) in the UVRR spectrum and shows a downshift of 21 cm\(^{-1}\) upon deuteriation. This downshift suggests the contribution of N7H str to this mode. This also confirms that at higher pH, N7H remains intact whereas proton is lost from the N1 position. Further, in 9DAHH\(^+\), the band appears at 1373 cm\(^{-1}\) and downshifts to 1334 cm\(^{-1}\) upon deuteriation. The upshift from neutral to protonated 9DAH is reproduced by the computed wavenumbers of neutral and protonated forms. Calculated assignments predict that at lower pH in protonated form, N1H bending decouples from the remaining band. The inherent wavenumber of C–N str lies in the region of 1400–1600 cm\(^{-1}\) (Deng, Callender, & Dale, 2000; Deng, Huang, Groesbeek, Lugtenburg, & Callender, 1994). Thus after decoupling of N1–H, primary C–N str in the protonated form upshifts with respect to the neutral position.

C–H bending mode in pyrrole ring

The band at 1291 cm\(^{-1}\) is attributed to the C8HC9 and C8C9H bends along with a minor contribution from N1–C6 str. This band downshifts by 34 cm\(^{-1}\) upon deuteriation in neutral 9DAH. In 9DAH\(^+\), this band appears at 1243 cm\(^{-1}\) and further downshifts to 1220 cm\(^{-1}\) upon H→D substitution. The isotope-induced downshift despite the loss of proton at N1 position implies reorganization of the mode to include N7–H contribution. Calculations show that the composition of the normal mode in the deprotonated form is different from that of the neutral molecule. The modified mode includes N1–C2–N3, C4–C5–N7 bends in addition to C8HC9 and C8C9H bends. A minor contribution from C2–N3 str is also observed. In 9DAHH\(^+\), the band appears at 1286 cm\(^{-1}\). This band downshifts to 1256 cm\(^{-1}\) (30 cm\(^{-1}\)) upon deuteriation. Predicted wavenumbers for protonated 9DAH also suggest an isotope-induced downshift of 21 cm\(^{-1}\).

N7–H bending mode

The band at 1236 cm\(^{-1}\) in the UVRR spectrum of neutral 9DAH is attributed to N7HC8 and C8C9H bends.

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**Table 5.** Experimental resonance Raman shifts of deprotonated 9-deazahypoxanthine in water and D\(_2\)O at pH 12.5 and computed (\(\text{wB97XD/6-31G (d, p)}\)) vibrational wavenumbers of deprotonated 9-deazahypoxanthine with mode assignments\(^a\).

| UVRR in H\(_2\)O | DFT | Mode assignments | UVRR in D\(_2\)O | DFT | Mode assignments |
|------------------|-----|-----------------|------------------|-----|-----------------|
| 1663             | 1732| Str C6O6 (69%)  + Be N1C6C2 (11%) | 1664             | 1732| Str C6O6 (69%)  + Be C6N1C2 (10%) |
| 1598             | 1633| Str C2N3 (13%)  + Str C8C9 (10%) + Str C4C5 (24%) + C2H + N7H | 1595             | 1628| Str C2N3 (115) + Str C8C9 (12%) + Str C4C5 (29%) |
| 1518             | 1605| Str C2N3 (12%)  + Str N3C4 (11%) + Be C4C5N7 (32%) | 1505             | 1601| Str C2N3 (20%) + Str N1C6 (10%) + Be C4C5N7 (30%) |
| 1531             | 1469| Str C4C5 (~11%) + Str C5N7 (23%) + Be N7C8C9 (15%) + Be N7HC8 (12%) | 1532             | 1501| Str C4C5 (~24%) + Be C2HN3 (20%) + Be C4C5N7 (14%) |
| 1479             | 1546| Str C8C9 (37%)  + Be C8HC9 (17%) + Be C2HN3 (13%) | 1471             | 1543| Str C8C9 (39%) + Be C8HC9 (19%) + Be C2HN3 (105) + Be C5N7C8 (10%) |
| 1424             | 1513| Str C4C5 (~16%) + Str N7C8 (11%) + Be C2HN3 (20%) | 1391             | 1448| Str N7C8 (~10%) + Str C5N7 (31%) + Be N7C8C9 (13%) |
| 1445             | 1405| Str N1C2 (35%)  + Str N1C6 (~10%) + Be C2HN3 (~17%) | 1421             | 1394| Str N1C2 (38%) + Str N7C8 (11%) + Be C2HN3 (~16%) |
| 1368             | 1413| Str C2N3 (25%)  + Be C2HN3 (33%) | 1367             | 1410| Str C2N3 (26%) + Str N3C4 (~11%) + Be C2HN3 (26%) |
| 1341             | 1325| Str N1C2 (11%)  + Str N3C4 (18%) + Str N7C8 (~17%) + Str N1C6 (~15%) + Str C5N7 (~12%) | 1320             | 1319| Str N3C4 (~18%) + Str N7C8 (22%) + Str N1C6 (13%) |
| 1243             | 1261| Str C2N3 (~10%) + Be N7HC8 (12%) + Be C8HC9 (25%) + Be C8C9H (~13%) + Be C4C5N7 (11%) + Be N1C2N3 (10%) | 1220             | 1249| Str C2N3 (~11%) + Str N7C8 (~16%) + Be C8HC9 (21%) + Be N1C2N3 (11%) |
| 1309             | 1208| Str C5N7 (~10%) + Be N7HC8 (18%) + Be C5N7C8 (11%) | 1300             | 1169| Be C8HC9 (15%) + Be C8C9H (~12%) + Be C5N7C8 (14%) |

\(^a\)Abbreviations: DFT, Density functional theory; Str, Stretch; Be, Bend; Py, Pyrimidine; Sci, Scissors; Pu, Purine.
in pyrrole ring. This band appears at 1309 and 1245 cm\(^{-1}\) in deprotonated and protonated 9DAH, respectively. Upon deprotonation, this mode decouples from N7HC8 bend in all three species. Deprotonation results in the reorganization of this mode, and the reorganized mode comprised of C5–N7–C8 bend in all three species.

**Assessment of the performance of DFT**

Here, we assess the performance of DFT (wB97XD/6–31G (d, p)) in predicting vibrational wavenumbers and H→D induced isotopic shifts in nucleobases. We note that the experiments are carried out in solution whereas the calculations are conducted *in vacuo*. DFT is one of the most widely used and cost-effective procedures to determine vibrational spectra of molecules. The hybrid functional wB97XD which accounts for dispersive forces has been shown to predict the compact structural parameters well (Minenkov, Singstad, Occhipinti, & Jensen, 2012). Since dispersion also affects chemical bonds and use of such a functional minimizes the general overestimation of bond order. These parameters have been found to differ from those predicted by B3LYP (Minenkov et al., 2012). Since a correct structure is a prerequisite to calculate vibrational frequencies, we chose wB97XD hybrid functional to determine the structural parameters and vibrational frequencies.

In the results presented here, we observed that the H→D induced isotopic shift is more reliably reproduced than the actual wavenumbers. Furthermore, the isotopic shifts in the region from 1100 to 1500 cm\(^{-1}\) in 9DAG as well as 9DAH. In particular, an exceptionally huge difference in the –NH2 scissoring mode at in neutral 9DAG is

| UVRR in H\(_2\)O | DFT | Mode assignments | UVRR in D\(_2\)O | DFT | Mode assignments |
|------------------|-----|------------------|------------------|-----|------------------|
| 1730             | 1917 | Str C6=O (77%) + Str C5C6 (14%) | 1713             | 1911 | Str C6=O (80%) + Str C6C5 (13%) |
| 1658             | 1733 | Str C2N3 (34%) + Str N1C2 (16%) + Be N1C2H (14%) + Be N1H + C2H + N7H | 1625             | 1719 | Str C2N3 (35%) + Str N1C2 (20%) + Be N1C2H (16%) |
| 1543             | 1639 | Str N1C2 (19%) + Str N3C4 (18%) + Be N3HC2 (28%) | 1509             | 1610 | Str C4C5 (26%) + Str N3C4 (30%) |
| 1599             | 1616 | Str C2N3 (10%) + Str C8C9 (25%) + Str N3C4 (10%) + Be N7HC8 (11%) | 1558             | 1687 | Str N1C2 (14%) + Str C8C9 (14%) + Str C4C5 (11%) + Be N1C2H (10%) + Be C8HC9 (11%) + Be C4C5N7 (17%) |
| 1444             | 1572 | Str C4C5 (13%) + Str C5N7 (23%) + Be N7HC8 (11%) + Be C4C5N7 (12%) | 1421             | 1528 | Str C5N7 (15%) + Be N7HC8 (19%) + Be N1HC2 (18%) |
| 1451             | 1502 | Str C8C9 (28%) + Str N7C8 (12%) + Be C8HC9 (17%) | 1411             | 1475 | Str N7C8 (11%) + Be C8HC9 (15%) |
| 1324             | 1451 | Str C8C9 (13%) + Be N1HC2 (16%) + Be C8HC9 (10%) + Be C2H + Be N1H | 1304             | 1413 | Str N1C2 (25%) + Be N1C2H (33%) + Be C2H + Be N1D |
| 1371             | 1330 | Be C2N3H (28%) + Be N1HC2 (11%) + Be C2H + Be N1H | 1292             | 1345 | Str C8C9 (11%) + Be C8HC9 (21%) + Be C8HC9 (13%) |
| 1373             | 1395 | Str C5N7 (18%) + Str N7C8 (20%) + Be N7HC8 (18%) | 1286             | 1302 | Str C8C9 (11%) + Be N3C4 (12%) + Be C8HC9 (21%) + Be C8HC9 (13%) |
| 1286             | 1324 | Str C8C9 (11%) + Be N3C4 (12%) + Be C8HC9 (21%) + Be C8HC9 (13%) | 1256             | 1281 | Str C8C9 (12%) + Be C8HC9 (26%) + Be C8HC9 (11%) |
| 1245             | 1264 | Str N7C8 (11%) + Be N7HC8 (13%) | 1174             | 1188 | Str C2N3 (23%) + Str N1C2 (27%) + Be C2N3H (12%) + Be N1HC2 (21%) |
| 1174             | 1188 | Str C2N3 (23%) + Str N1C2 (27%) + Be C2N3H (12%) + Be N1HC2 (21%) | 1121             | 1137 | Str N7C8 (29%) + Be N7HC8 (21%) + Be C8HC9 (18%) |
| 1073             | 1098 | Str C8C9 (18%) + Be C8HC9 (20%) + Be C8HC9 (51%) | 1198             | 1201 | Be C8HC9 (15%) + Be C5N7C8 (13%) |

*Abbreviations: DFT, Density functional theory; Str, Stretch; Be, Bend; Py, Pyrimidine; Sci, Scissors; Pu, Purine.*
observed where a computed isotopic downshift of 397 cm$^{-1}$ (1655–1258 cm$^{-1}$) is predicted against an observed isotopic shift of 217 cm$^{-1}$ (1608–1391 cm$^{-1}$). This indicates the limitations of DFT in prediction of the normal mode composition accurately. A lower magnitude of isotope-induced shift in observed spectra suggests that the in D$_2$O spectrum, the band at 1391 cm$^{-1}$ retains the contribution from –ND$_2$ moiety more than that predicted by DFT (32%). Further, we have obtained all the calculated wavenumbers under harmonic approximation. The substantial differences in the higher wavenumber region between experiment and calculations are attributed to the anharmonicity of the system. In cytosine and uracil, it has been reported that even higher level DFT calculations done under in vacuo conditions predict erroneous wavenumbers (Shanmugasundaram & Puranik, 2009). The wavenumbers predicted above 1300 cm$^{-1}$ are overestimated by more than 80 cm$^{-1}$. Incorporation of PCM model did not improve the results. Despite their limitations, DFT methods are by far the most widely used methods to predict the normal mode assignments which are pre-requisite for using resonance Raman spectroscopy as a probe to report on the nucleobase–protein interaction.

Conclusion

The systematic observation of purine spectra and their assignments to normal mode vibrations in this study provides the basis to examine these molecules in their enzyme-bound forms. In 9DAG and GMP, the neutral species bears a proton at N1 position. It has been shown that at higher pH, the proton from N1 position leaves to form 9DAG$^-$ and GMP$^-$. At lower pH, 9DAG is protonated at N3 whereas GMP gains a proton at N7 position. In Hx, first deprotonation from N7 of purine ring occurs at pH 10 and the second deprotonation occurs from N1 position at pH 12.5, whereas in 9DAH, deprotonation at N1 is observed at pH 10. Deprotonation at N7 of purine ring is not observed in 9DAH until pH 12.5. At low pH, 9DAH is protonated at N1, N3, and N7 positions. The protonated forms of Hx bears proton at N1, N7, and N9 and in IMP at N1 and N7 of the purine rings. Raman shifts in carbonyl stretch mode follow exactly same pattern across different pH in 9DAG/GMP and 9DAH/IMP in H$_2$O and D$_2$O. The purine base analogues studied here exist in C6=O keto tautomers in solution at all pH. The effect of protonation and deprotonation is well captured by the carbonyl stretching mode which is why it is regarded as the marker band of protonation state. Furthermore, the bands comprised of NH vibrations are regarded as suitable marker bands for hydrogen bonding interaction at the active site of enzyme. In general, pyrimidine and pyrrole ring modes are observed as of higher intensity and thus can serve as a probe which can report on the local environment change of the ligand. In majority of the bands, isotope-induced shifts observed in UVRR spectra are well reproduced in the DFT calculations. These isotope-induced shifts have been used as an important criterion for the normal mode assignments of UVRR bands in the solution state. Further, calculated Raman shifts upon protonation and deprotonation show similar trend as that of observed shifts. These results corroborate the assignments of the observed protonation state at lower and higher pH.

This study has the direct implication on the structural studies of PNP and HGPRT catalysis which are the integral enzymes of purine salvage pathway. Since de novo nucleotide synthesis pathway is absent in *Plasmodium falciparum*, Hx derived from the salvage pathway is the sole source of purines in these parasites. Hence, PNP and HGPRT are considered as the potential targets of anti-malarials. In this regard, elucidating the solution structure and protonation state in potent nucleobase inhibitors is indispensable in any type of structural studies. Further, it will help in future vibrational studies of the nucleobase analogues at the active site of enzyme.

Supplementary material

DFT (B3LYP/6–31G (d, p)) computed spectra of neutral, N3 protonated, N1 deprotonated, N7 deprotonated 9DAG and 9DAH. Normal mode vectors of 9DAG and 9DAH. Lorentzian fits to a few bands in the UVRR spectra of 9DAG and 9DAH. The supplementary material for this paper is available online at [http://dx.doi.10.1080/07391102.2015.1042916](http://dx.doi.10.1080/07391102.2015.1042916).

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