Solution structures of purine base analogues 6-chloroguanine, 8-azaguanine and allopurinol

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Analogues of purine bases are highly relevant in the biological context and have been implicated as drug molecules for therapy against a number of diseases. Additionally, these molecules have been implicated to have a role in the prebiotic RNA world. However, experimental data on the structures of these molecules in aqueous solution is lacking. In this work, we report the ultraviolet resonance Raman spectra of 6-chloroguanine, 8-azaguanine and allopurinol, obtained with 260 nm excitation. The reported spectra have been assigned to normal modes computed from density functional theory (B3LYP/6-31G (d,p)) calculations. This work has been useful in identifying the solution-state structures of these molecules at neutral pH. We find that the guanine analogues 6-chloroguanine and 8-azaguanine exist as keto-N9H and keto-N7H tautomers in solution, respectively. On the other hand, the hypoxanthine analogue allopurinol exists as a mixture of keto-N9H and keto-N8H tautomers in solution. We predict that this work would be particularly useful in future vibrational studies where these molecules are present in complexes with their target proteins.

Keywords: ultraviolet resonance Raman spectroscopy; density functional theory; 6-oxopurines

Introduction

Cellular DNA is composed of heterocyclic purines and pyrimidines. Interestingly, it has been found that metabolism of purines in several disease-causing protozoan parasites is remarkably different from that in humans, whereas pyrimidine metabolism is similar (Berens, Krug, & Marr, 1995). As a result, enzymes in the purine salvage pathway have been identified as potential drug targets against these parasites. The possibility of using structural analogues of purine bases that might inhibit enzymes in the salvage pathway (Berens et al., 1995; Eakin, Guerra, Foca, Torres-Martinez, & Craig, 1997; Freymann et al., 2000), or get incorporated into their DNA resulting in cellular toxicity (Keough et al., 2006), is currently under active investigation. A detailed understanding of the structures of these analogues is a prerequisite for any biochemical or biophysical investigations on their protein complexes. The current study is aimed at comprehending the structures of three such biologically relevant purine base analogues.

Vibrational spectra of molecules are highly useful in identifying their structure and predominant tautomeric states. Further, these spectra are sensitive to changes in the local environment of molecules and can be used to report on the same. We have previously reported the ultraviolet resonance-enhanced Raman spectra (UVRR), using 260 nm excitation, of the purine bases hypoxanthine, guanine and xanthine (and/or their corresponding nucleotides), with detailed normal mode assignments (Gogia, Jain, & Puranik, 2009). The vibrational normal modes were computed using exhaustive density functional theory (DFT) calculations on possible tautomers. In the current report, we extend this previous work to include the purine base analogues 6-chloroguanine, 8-azaguanine and allopurinol using 260 nm excitation.

As shown in (Figure 1(a)), 6-chloroguanine is a structural analogue of guanine with a chlorine atom attached at C6 in place of the oxo group present in guanine. Caddell et al. (2004) have shown that alkyl derivatives of 6-chloroguanine are highly useful in the synthesis of adenosine analogues used in therapy of inflammatory diseases.
molecule can replace guanine in RNA resulting in cellular toxicity (Bergquist & Matthews, 1962; Rivest, Irwin, & Mandel, 1982a). The incorporation of 8-azaguanine in mRNA of tumor cells has been found to inhibit protein synthesis (Zimmerman & Greenberg, 1965). As a result, 8-azaguanine has been implicated as a lead molecule in cancer therapy (Nelson, Carpenter, Rose, & Adamson, 1975; Rivest, Irwin, & Mandel, 1982b). Also, 8-azaguanine is used to identify, differentiate and inhibit pathogenic leptospira (Johnson & Harris, 1968; Johnson & Rogers, 1964; Ridzlan, Bahaman, Khairani-Bejo, & Mutalib, 2010). Both 8-azaguanine and 6-chloroguanine have been found to be efficient substrates for Plasmodium falciparum hypoxanthine guanine phosphoribosyl transferase and have been proposed as potential lead compounds for drugs against malaria (Keough et al., 2006).

Allopurinol is a structural analogue of hypoxanthine where the atoms at the seventh and eighth positions in the purine ring are interchanged. This molecule is used as a xanthine oxidase inhibitor in patients suffering from gout (Pacher, Nivorozhkin, & Szabó, 2006) and as an oxygen scavenger in patients undergoing coronary angioplasty (Guan et al., 2003; Rajendra et al., 2011; Stone, 2011). Allopurinol is also used in preventing tumor lysis in certain types of cancer (Cairo & Bishop, 2004; Holdsworth & Nguyen, 2003). Use of allopurinol as a chemotherapeutic agent against Chagas disease has also been implicated (Marr & Berens, 1988).

Materials and methods

6-chloroguanine, 8-azaguanine and allopurinol were purchased from Sigma Co. and used without further purification. Stock solutions of the purine bases were prepared in 0.4 N NaOH. Samples for UVRR spectroscopy were prepared by diluting the stocks to 0.5 mM in 20 mM potassium phosphate buffer at pH 7.0. Optical setup used for laser Raman experiments was the same as described previously (Gogia et al., 2009; Jayanth, Ramachandran, & Puranik, 2009). In short, the third harmonic ($\lambda_{exc} = 260$ nm) from a tunable Ti:Sapphire laser (Indigo-S, Coherent, Inc.) was impinged on a rotating NMR tube containing the sample solution. Light scattered at an angle of 135° was collimated and focused on a monochromator (Jobin-Yvon) fitted with a 3600 grooves/mm diffraction grating. Spectra were recorded using a CCD camera (Jobin-Yvon). All spectral processing was done using the software SynerJY (Jobin-Yvon), which is a version of Origin 7.0.

All the reported DFT calculations were carried out with the B3LYP (Becke, 1993; Lee, Yang, & Parr, 1988) functional and the 6–31G(d,p) basis set using the Gaussian03 suite (Frisch et al., 2003). The computed wave-numbers were scaled using a uniform scaling factor as described previously (Gogia et al., 2009). Bands ascribed to carbonyl stretching vibrations were scaled by a factor of 0.93 (Gogia et al., 2009). Theoretically predicted normal modes were visualized using the software Chemcraft (http://www.chemcraftprog.com). Potential energy distributions of the computed normal modes were calculated using the software VEDA (Jamróz, 2004).

Structure of 6-chloroguanine at neutral pH

The UVRR spectrum of 6-chloroguanine at pH 7.0, obtained with 260 nm excitation, is shown in Figure 2(a). This spectrum is found to be remarkably similar, in intensity ratio as well as band positions, to the UVRR spectrum of neutral guanosine monophosphate (GMP) reported earlier (Gogia et al., 2009). This suggests that the structure of 6-chloroguanine in solution is homologous to the structure of GMP in solution. Since GMP has a sugar group attached at the N9 position, the tautomer of 6-chloroguanine analogous to neutral GMP should contain a hydrogen atom at this position (see Figure 1(a)). The DFT (B3LYP/6–31G(d,p)) calculations were carried out to determine the structure of this tautomer followed by computation of the vibrational spectrum. We find that the computed spectrum agrees well with the experimental spectrum. On the basis of these results, we ascertained that 6-chloroguanine exists as the N9H tautomer at neutral pH (shown in Figure 1(a)). Detailed normal mode assignments of the spectrum shown in Figure 2(a) are given in Table 1.
Computed parameters of this molecule corresponding to the minimum energy structure are listed in Table 1, supporting information. We have previously reported detailed assignments of UVRR spectra of various protonated states of GMP. The assignments reported here were arrived at using the prior assignments as a starting point. In the following, we discuss some important vibrational bands of 6-chloroguanine. Further, we predict the sensitivity of these bands to changes in the structure of this molecule that might result from binding to proteins.

**1600–1400 cm⁻¹ region**

The 1606 cm⁻¹ band of 6-chloroguanine has been assigned to a scissoring vibration of the exocyclic NH₂ group. This band appears at 1602 cm⁻¹ in GMP and guanine (Giese & McNaughton, 2002; Gogia et al., 2009). This is the second most intense band in the 6-chloroguanine spectrum and can serve as a marker for hydrogen bonding interactions formed with the NH₂ moiety. The medium-intensity band at 1585 cm⁻¹ in the UVRR spectrum of 6-chloroguanine corresponds to the 1576 cm⁻¹ band of GMP (Gogia et al., 2009) and is attributed to pyrimidine ring vibrations (see Table 1). Since this band arises almost exclusively due to pyrimidine ring vibrations, we predict that this band would be sensitive to any structural change in the ring that might arise as a result of binding to protein.

The 1542 cm⁻¹ band in the UVRR spectrum of 6-chloroguanine corresponds to the 1537 cm⁻¹ band of GMP (Gogia et al., 2009) and arises due to imidazole ring (N7–C8) vibrations coupled to C8–H bending. This band will serve as a marker for structural perturbations in the imidazole ring.

The most intense band in the 6-chloroguanine UVRR spectrum is observed at 1480 cm⁻¹. In GMP (Gogia et al., 2009), this band is observed at 1486 cm⁻¹. This

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Table 1. Normal mode assignments of the UVRR spectrum of 6-chloroguanine obtained with 260 nm excitation. Corresponding bands of neutral GMP (Gogia et al., 2009) have been listed for comparison.

| UVRR       | DFT      | Normal mode assignment |
|------------|----------|------------------------|
| GMP 6-CIG  | 6-CIG    | St N1C6(20%)--N3C4(19%)--N3C4C5(−11%) |
| 1602 1606  | 1614     | Sci NH2(62%)+St N2C2(23%) |
| 1576 1585  | 1567     | St N3C4(−10%)--N3C2(−17%)--C2N3C4(10%)--C4N9C8(15%)+Be NH2a |
| 1537 1542  | 1521     | St N7C8(41%)+Be C8H(−14%)+Sci NH2 |
| 1486 1480  | 1476     | Sci NH2(−14%)+St N2C2(15%)--N1C2N3(14%) |
| 1414 1407  | 1407     | St N1C6(36%)--N2C2(−13%)--N9C4(13%)+Be NH2b |
| 1365 1381  | 1386     | Be N9H(17%)+C8H+St N3C4(10%)--C2N3C4(11%) |
| 1331 1353  | 1359     | Be N9H (−27%)+Rock NH2 (−10%)+St N7C5(−13%)--N9C8(14%) |
| 1318 1309  | 1321     | Be C8H (18%)+St N1C2(−11%)--N7C5(−12%)--C5N7C8(17%)--N9C8N7(−11%)+Rock NH2 |
| 1292 1292  | 1292     | St N7C8(−17%)--N1C6(11%)--N3C4(−18%)+Be C8H(−17%)+N9H |
| 1203 1214/1191 | 1184 | Be C8H(30%)+St N7C5(19%)--C5C6--C4N9 |
| 1104      | 1085     | Rock NH2(44%)+St N3C2(−22%) |
|           | 1054     | St N9C8(48%)+Be N9H(34%) |

*aScaling factor used: 0.98.*
band is assigned to a mode comprising of NH$_2$ scissoring and C2–N2 and N1–C2–N3 vibrations. It has been seen in previous work that this mode is perturbed when hydrogen bonding interactions are made with GMP (Gogia, Balaram, & Puranik, 2011) via the NH$_2$ group. Based on this data and the high relative intensity of this mode, we predict that this mode would be highly useful in determining interactions of this group of 6-chloroguanine in protein complexes.

The 1407 cm$^{-1}$ band of 6-chloroguanine has been assigned to purine ring vibrations as shown in Table 1 and corresponds to the 1414 cm$^{-1}$ band of GMP (Gogia et al., 2009).

**1400–1100 cm$^{-1}$ region**

The various low intensity bands in the 1400–1100 wavenumber region of 6-chloroguanine arise primarily as a result of various bending vibrations of the ring substituents as listed in Table 1. Bending vibrations are known to be intrinsically weak in Raman spectra. Of these, the band at 1292 cm$^{-1}$ is the most intense, and is attributed to imidazole ring vibrations coupled to C8–H bending.

**Structure of 8-azaguanine at neutral pH**

The UVRR spectrum of 8-azaguanine obtained using 260 nm excitation is given in Figure 2(b). Vibrational spectra of 8-azaguanine have been reported previously and the spectrum shown here agrees with previous data (Pandey, Asthana, & Mishra, 1993) and further, extends the information obtained from this data. In this work, a normal Raman spectrum of 8-azaguanine was reported which covered a wavenumber range from 1400 to 100 cm$^{-1}$. This spectrum had a large fluorescence background such that low-intensity bands were not observed. The resonance-enhanced Raman spectrum of 8-azaguanine reported in this work has a much improved signal-to-noise ratio such that even the low-intensity bands can be seen. Moreover, this spectrum covers a wavenumber range of 1800–1100 cm$^{-1}$. X-ray crystallography on 8-azaguanine monohydrate has shown that this molecule exists as the keto-N1H, N9H tautomer in the crystalline state (Macintyre, Singh, & Werkema, 1965). On the other hand, theoretical studies have predicted that 8-azaguanine should exist as a mixture of keto-N1H, N7H and keto-N1H, N9H tautomers in solution with the N7H tautomer having lower energy (Contreras & Madariga, 1998). We have previously shown that hypoxanthine exists as a mixture of keto-N1H, N7H and keto-N1H, N9H tautomers at neutral pH (Gogia et al., 2009). This was concluded on the basis of comparison of the obtained experimental UVRR spectrum of this molecule with the spectra computed for both these tautomers from DFT calculations. We found that the experimental spectrum arose from both N7H and N9H tautomers. Moreover, most of the observed bands appeared as doublets since bands of the two tautomers appeared at small relative shifts. These features are absent in the UVRR spectrum of 8-azaguanine (Figure 2(b)) suggesting that only a single tautomer predominates in solution. Comparison of this spectrum with the UVRR spectrum of neutral GMP can resolve the nature of the tautomer present in solution (since GMP serves as a model compound for the presence of N9=H). We find that the 8-azaguanine spectrum differs from the previously reported UVRR spectrum of GMP (Gogia et al., 2009). Moreover, we find that the spectra computed using DFT for the 8-azaguanine tautomer containing N9H does not agree with the observed UVRR spectrum. On the basis of these observations, we have ruled out the existence of the N9H tautomer of 8-azaguanine in solution at neutral pH. Following this analysis, we compared the computed vibrational spectrum of the keto-N1H, N7H tautomer of 8-azaguanine with the UVRR spectrum of 8-azaguanine (shown in Figure 1(b)). The predicted vibrational spectrum of the N7H tautomer correlates very well with the experimental spectrum (see Table 2). Moreover, we note that the intensity pattern of this spectrum is similar to that of neutral xanthine (Gogia et al., 2009), especially in the 1200–1350 wavenumber region, which also exists as the N7H tautomer. Based on these results, we deduced that 8-azaguanine exists as the keto-N1H, N7H tautomer in aqueous solution at neutral pH (shown in Figure 1(b)). In the following, we discuss assignments of some key Raman bands observed in the spectrum of 8-azaguanine. Detailed normal mode assignments of the UVRR spectrum of 8-azaguanine are given in Table 2. Calculated interatomic bond lengths and partial atomic charges of 8-azaguanine are given in Table 1, supporting information.

**Carbonyl stretching mode**

The low intensity band at 1699 cm$^{-1}$ in 8-azaguanine has been assigned to the carbonyl stretching vibration. In neutral xanthine, the corresponding band is observed at 1693 cm$^{-1}$ (Gogia et al., 2009). It is expected that this band will serve as a marker of the hydrogen bonding status of the 6-oxo group in complexes of 8-azaguanine bound to proteins.

**1600–1400 cm$^{-1}$ region**

The high intensity bands observed in the 1600–1400 cm$^{-1}$ region of the UVRR spectrum of 8-azaguanine are assigned to various purine ring stretching vibrations as listed in Table 2. The 1609 and 1540 cm$^{-1}$ bands have been ascribed to NH$_2$ scissoring vibrations, and can serve as markers for interactions of the amino group when 8-azaguanine is complexed with protein. The medium intensity bands observed at 1540 and
Table 2. Normal mode assignments of UVRR spectrum of 8-azaguanine obtained with 260 nm excitation. Corresponding bands of neutral xanthine (Gogia et al., 2009) have been listed for comparison.

| UVRR | DFT* | Normal Mode Assignment |
|------|------|------------------------|
| Xan  | 8-azaG | 8-azaG |
| 1693 | 1699 | 1690 |
| 1604 | 1609 | 1617 |
| 1540 | 1574 | 1572 |
| 1477 | 1551 | 1546 |
| 1454 | 1464 | 1470 |
| 1416 | 1378 | 1370 |
| 1331 | 1356 | 1322 |
| 1283 | 1269 | 1244 |
| 1208 | 1220 | 1230 |
| 1132 | 1156 | 1160 |
| 1121 | 1097 | 1093 |
| 1036 | 1083 | 1047 |

*Scaling factor used: 0.99.

1522 cm⁻¹ have been attributed to imidazole ring vibrations, and can be used as probes to study changes in the local environment of the imidazole ring.

**1400–1200 cm⁻¹ region**

In the 1400–1200 cm⁻¹ region of 8-azaguanine, three high intensity bands are observed at 1356, 1269 and 1208 cm⁻¹. The band at 1356 cm⁻¹ is ascribed predominantly to N1–H bending vibrations. Thus, this band can be used as a marker for hydrogen bonding interactions made with this group. The 1269 and 1208 cm⁻¹ bands arise primarily due to vibrations of the N7–N8 bond. 8-azaguanine is distinct from the guanine ring only in having a nitrogen atom at the eighth position. The high intensity bands arising due to the N7–N8 vibrations can be used to report on local changes around this bond when 8-azaguanine is bound to proteins. Moreover, a comparison of those with obtained with unsubstituted guanine can provide information on the differences in interactions, resulting from the C8 to N8 replacement, if any.

**Structure(s) of allopurinol at neutral pH**

The UVRR spectrum of allopurinol at pH 7.0 is given in Figure 2(c). A preliminary observation of this figure reveals that many bands in this spectrum appear as doublets or shoulders to bands of higher intensity. These features are consistent with the spectrum with neutral hypoxanthine which has a mixture of two tautomers present in solution (Fernandez-Quejo, De La Fuente, & Navarro, 2005; Gogia et al., 2009). This indicates that allopurinol exists as a mixture of tautomers at neutral pH.

Theoretical calculations on allopurinol have indicated that it should exist as a mixture keto-N9H and keto-N8H (N9H/N7H) tautomers in solution (Costas & Acevedo-Chavez, 1999; Hernández, Luque, & Orozco, 1996) Figures (1(c) and (d)), analogous to the N9H/N7H tautomerism observed in hypoxanthine. The calculated difference in the free energies of the two tautomers is reported to 3.6–4.9 kcal/mol, depending on the basis set used, with the keto-N9H tautomer having lower energy. Coexistence of these two tautomers in DMSO has been detected by 13C NMR spectroscopy (Babushkina, Leonova, Chernyshev, & Yashunskii, 1980) and in the solid-state by vibrational spectroscopy – both Raman and FTIR (Torreggiani, Tamba, Trinchero, & Fini, 2003). X-ray diffraction methods have revealed that allopurinol exists as the keto-N9H form in crystals (Prusiner & Sundaralingam, 1972). Shukla and Mishra have reported fluorescence spectra of allopurinol (Shukla, 1996). Comparison of these spectra with computed (MNDO; AM1; CNDO/s-Cl) electronic transition energies predicted for various tautomers suggested that the enol form (containing N9H) of allopurinol is predominant in aqueous solution and the keto tautomer is only partially populated (Shukla, 1996). Considering all this data, we evaluated the possibility that the UVRR spectrum of allopurinol represented presence of both, the keto-N9H/N8H and enol-N9H forms, in order to determine the ones present in solution. We find that the solution-state UVRR spectrum of allopurinol shown in Figure 2(c) corresponds to the computed spectra of keto-N9H and keto-N8H tautomers of allopurinol (shown in Figures 1(c) and (d), respectively). Bands corresponding to the enol tautomer were not observed. Thus, we conclude from this analysis that allopurinol exists predominantly as a mixture of the keto-N8H/N9H tautomers in aqueous solution at neutral pH. The normal mode assignments of the observed UVRR spectrum of allopurinol are summarized in Table 3, and a detailed discussion is provided below. Structural parameters calculated for the two tautomers of allopurinol are listed in Table 2, supporting information.
Table 3. Normal mode assignments of UVRR spectrum of allopurinol obtained with 260 nm excitation. Corresponding bands of neutral hypoxanthine N7H/N9H tautomers (Gogia et al., 2009) have been listed for comparison.

| UVRR DFT* | Normal mode assignment |
|-----------|------------------------|
| Hx Allo  | Allo N9H N8H           |
| 1694 1697| St C6O (76%)+St C6N1C2(-12%) |
| 1620    | St C6O(77%)+Be C6N1C2(-10%) |
| 1596 1607| St N3C2(57%)-C5C6+Be C2H+N1H |
| 1575 1578| St C5C7(39%)-N9C4(-24%)-N3C2+Be C7H+N8H+C2H+N1H |
| 1541 1552| St C5C4(-24%)-N3C4(21%)-N9C4(-10%)+Be N9H(-17%) |
| 1512 1522| St N8C7(13%)-C5C4(-10%)-C5C4N9(-16%)-N3C4C5(12%)+Be C7H(-11%)+N1H+C2H |
| 1464 1475| St N3C4(27%)-C5C7N8(-11%)+Be C2H+N1H+C7H+N8H |
| 1452 1446| St N8C7(21%)+Be N1H(-15%)+N9H(-13%)+C2H+C7H |
| 1424    | Be N1H(37%)+St C5C7(14%)-N9C4(13%)-N1C2(-11%) |
| 1396 1400| Be N1H(-18%)+St C5C4(-18%)-N9C4(20%) |
| 1396 1400| St N9C4(26%)-St C5C7(11%)+Be N1H(-13%)+C2H |
| 1367 1378| St N8C7(21%)-N9C4(10%)-C4N9N8(11%)+St N1C2(-10%)+Be N1H(13%) |
| 1351 1371| Be C2H(56%)+St N3C4-C5C6 |
| 1338 1336| Be C2H(57%)+St N3C2(12%) |
| 1270 1297| St N8C7(30%)-C5C7N8(13%)+Be C7H(11%)+C7H+N8H |
| 1248    | Be N9H(43%)+C2H+St N9C4(-14%) |
| 1219 1222| Be C7H(-18%)+St N9N8(-13%)-C5C7(-11%)-N1C2 |
| 1192 1207| Be C7H(24%)+St C2N3C4(13%)-C5C6 |
| 1178    | Be N8H(-23%)+St N9N8(-18%)-N8C7(12%)-C5C7N8(-11%)-N1C6(-10%) |
| 1149 1115| St N1C2(38%)+Be N1H(28%) |
| 1107    | St N1C2(36%)+Be N1H(27%) |

*Scaling factor used: 0.98.

1600–1400 cm⁻¹ region

High intensity modes observed at 1620 and 1607 cm⁻¹ in the UVRR spectrum of allopurinol (Figure 2(e)) arise as a result of N3–C2 stretching and C2–H bending vibrations of the N8H and N9H tautomers, respectively, as given in Table 2. The band corresponding to the 1607 cm⁻¹ band of allopurinol appears at 1596 cm⁻¹ in neutral hypoxanthine (Gogia et al., 2009). These modes can serve as markers for pyrimidine ring interactions in complexes of allopurinol with proteins.

The bands at 1578 and 1525 cm⁻¹ are assigned to overall purine ring vibrations of the N8H and N9H tautomers, respectively. These bands correspond to the 1575 cm⁻¹ (N9H) and 1541 cm⁻¹ (N7H) bands of hypoxanthine. Imidazole ring vibrations give rise to low-intensity bands at 1505 cm⁻¹ (N9H tautomer) and 1475 cm⁻¹ (N8H tautomer). Corresponding bands in neutral hypoxanthine (N9H tautomer) are observed at 1512 and 1464 cm⁻¹. The 1620 and 1578 cm⁻¹ bands of allopurinol can be used as markers for the N8H tautomer, while the 1607 cm⁻¹ band is a marker of the N9H tautomer.

The medium-intensity band at 1445 cm⁻¹ in the allopurinol UVRR spectrum is assigned to N8–C7 stretching coupled to N1–H, N9–H bending vibrations of the N9H tautomer. This band can be used as a reporter of contacts made with the N1H and N9H groups of allopurinol by interacting proteins. In hypoxanthine, this band is observed at 1452 cm⁻¹ (N7H) (Gogia et al., 2009). In the N8H tautomer, the N8–C7 stretching vibration is downshifted remarkably to appear at 1336 cm⁻¹. The corresponding band at 1338 cm⁻¹ in neutral hypoxanthine is assigned to C8H bending, and is thus, different in mode composition from the one observed in allopurinol.

1400–1200 cm⁻¹ region

The high intensity band of allopurinol observed at 1400 cm⁻¹ has been ascribed to imidazole ring vibrations of both tautomers of allopurinol (N9H and N8H). This mode is among the two most intense bands in this spectrum, and can be used as marker of local interactions made with the imidazole ring of allopurinol. This mode is observed at 1396 cm⁻¹ (assigned to the N9H tautomer) in hypoxanthine.

C–H vibrations give rise to various low-intensity modes in the UVRR spectrum of allopurinol. While the 1378 and 1351 cm⁻¹ bands arise due to C2–H bending vibrations of the N8H and N9H tautomers, respectively, those at 1248 cm⁻¹ (N8H tautomer), 1222 cm⁻¹ (N9H tautomer) and 1192 cm⁻¹ (N9H tautomer) are ascribed to C7–H bending vibrations (see Table 3). Corresponding
bands in neutral hypoxanthine are observed at 1367 cm$^{-1}$ (N9H), 1270 cm$^{-1}$ (N9H) and 1219 cm$^{-1}$ (N7H/N9H).

Concluding remarks
Vibrational spectroscopy can provide a wealth of information on the structures of molecules in solution. The fact that the spectra of the three purine analogues are very different demonstrates high sensitivity and thus, high utility of UVRR in detecting structural difference in these analogues. Application of UVRR spectroscopy has allowed unambiguous identification of the structure that these molecules populate in solution, and revealed the fact that allopurinol populates more than one tautomeric form in solution. In this work, we have established the solution-state structures of three biologically relevant analogues of the purine bases guanine and hypoxanthine – 6-chloroguanine, 8-azaguanine and allopurinol – using ultraviolet resonance Raman spectroscopy coupled with DFT calculations. We provide detailed normal mode assignments of the obtained Raman bands to computed normal modes (Tables 1–3). Among the molecules studied, theoretical calculations of the ground-state structures of 8-azaguanine and allopurinol have been reported previously (Contreras & Madariaga, 1998; Costas & Acevedo-Chavez, 1999; Hernández et al., 1996). We have built on these studies to provide a detailed analysis of the vibrational spectra of these molecules.

It should be noted that the current spectra cannot be used to comment on aggregation or H-bond formation of these molecules. Information on H-bond formation may be collected by recording the spectra of these molecules in multiple solvents.

This work provides conclusive evidence that 6-chloroguanine exists as the N9H tautomer (Figure 1(a)), while 8-azaguanine exists predominantly in the N7H form (Figure 1(b)). Allopurinol exists as a mixture of its keto-N9H/N8H tautomers (Figures 1(c) and (d)) which is analogous to the keto-N7H/N9H tautomerism observed in hypoxanthine (Gogia et al., 2009). Further, we have identified marker bands in the observed spectra that can be used to report on hydrogen-bonding interactions that might form when these molecules are bound to their target proteins. This study provides important characteristic spectra and assignments and identifies tautomers for future investigations of these molecules in complexes with target proteins.

Significance
Analogue of purines have been identified as lead compounds for therapy against a number of diseases (Adachi, Motomatsu, & Yara, 1979; Alvarez, Lee, & Abel-Santos; 2010; Cairo & Bishop, 2004; Cassera, Zhang, Hazleton, & Schramm, 2011; Cunico et al., 2006; Hosaka, Suzuki, & Kumada, 2011; Keough et al., 2006; Ogilvie et al., 1984; Yang et al., 2010). Purine base analogues can also be used to identify interactions of individual functional groups in the natural substrate in the protein complex. For instance, de Koning and co-workers have used several nucleobase analogues in order to understand the interactions various nucleobase transporters make with their nucleobase substrates (Al-Salabi & de Koning, 2005; Wallace, Candlish, & De Koning, 2002). Recent work by Schramm and co-workers utilizing a number of immucillin analogues (analogue of nucleosides where the purine N9 is substituted with a carbon atom and the ribose oxo group is replaced a NH group) shows that change in a single hydrogen-bond interaction can cause a change of up to 10 kJ in binding energy (Kicska et al., 2002).

Analogue of purine bases are of interest for their possible role in theories of the origin of life (Joyce, 2002; Miller & Cleaves, 2006; Orgel, 2004). It is thought that these molecules existed as early as the prebiotic era (Levy & Miller, 1999). This theory is supported by the discovery of purine analogues in carbonaceous meteorites which are thought to have added to the prebiotic milieu (Callahan et al., 2011; Sephton, 2002). A recent review discusses how the various components of RNA – sugars, phosphates and bases – might be modified to give rise to different RNA configurations (Joyce, 2002). These included purine nucleobases that have a substituted functional group attached to the C6 and also, substitution at the C8 positions (Joyce, 2002). It has been hypothesized that the incorporation of these molecules in ribozymes allowed the population of various conformers through interactions with their altered functional groups (Lau & Unrau, 2009). Detailed knowledge of the solution structures of purine bases would be highly useful in order to understand how these alternate RNA conformations might be stabilized.

In the context of the various roles of purine base analogues in pharmacology and life sciences discussed above, detailed knowledge of the structures of these molecules in solution is the key to understanding their biological relevance. Yet, experimental structural data on these molecules and their tautomeric ratio in solution is scarce. The current study is an attempt at bridging this gap in knowledge.

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Supplementary material

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