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Vibronic spectra of protonated hydroxypyridines: contributions of prefulvenic and planar structures.

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Abstract

Various hydroxypyridine derivatives are endogenous or synthetic photosensitizers which could contribute to solar radiation damages. The study of their excited states could lead to a better understanding of their action mechanisms. We present here the ultraviolet (UV) spectra of the protonated 2-, 3- and 4-hydroxypyridine. These spectra were obtained with an experimental device coupling an electrospray ion source with a cold quadrupole ion trap and a time of flight mass spectrometer. They display well resolved vibrational structures, with a clear influence of the position of the OH group. These results are interpreted with excited states calculations at the coupled cluster CC2 level.

1 – Introduction

Because photochemistry underlies the function of many biomolecules and can initiate DNA damage and thus cause disease,^{1,2} there is significant interest in the UV photochemistry of aromatic heterocycles relevant to biology.³⁻⁵ The deactivation of excited DNA molecules may involve local, « monomer-like » processes,² which reinforces the importance of understanding the photochemistry of model molecules that mimic the molecular subunits comprising larger biologically relevant compounds. 2-, 3- and 4-hydroxypyridines are such model systems. Indeed, 2-hydroxypyridine exhibits two stable tautomeric forms: the enol form and the keto one called 2-

pyridone. These two tautomers are related by H atom transfer between N and O sites of the molecule and they are nitrogen heterocycles with the same H-bonding sites as uracil and thymine. These similarities make the mixed 2-pyridone 2-hydroxypyridine dimer a relevant model compound for tautomeric DNA-base pair analogues.⁶ As a consequence, gas phase spectroscopy of the neutral 2-hydroxypyridine in molecular beams has been extensively documented over the last decades.⁷⁻¹¹ Concerning 3-hydroxypyridine, its cation has been identified as a minimum phototoxic chromophore sufficient to effect skin cell sensitization¹² and 3-hydroxypyridine derivatives, comprising a wide range of skin biomolecules, are capable of skin photo-oxidative damage.¹³ This molecule is then more than a model system, and the study of its excited states could lead to a better understanding of its action mechanisms.

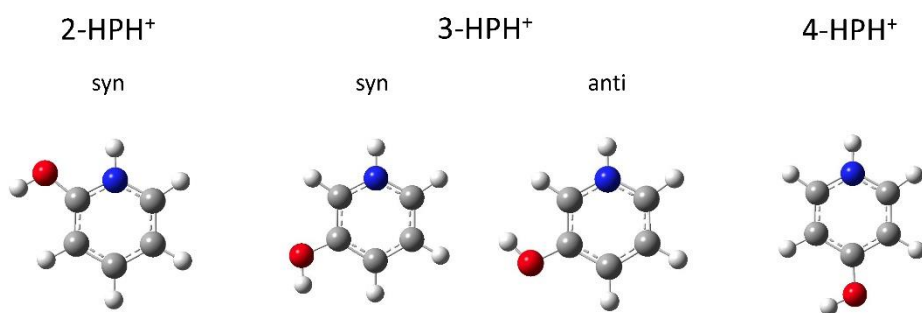


Chart 1: Structures of the three investigated protonated hydroxypyridines HPH⁺

We present here a systematic study of the influence of the position of the hydroxyl group in protonated hydroxypyridine molecules (see chart 1). Production and spectroscopic studies of protonated aromatic amino acids¹⁴⁻¹⁷ and of the protonated DNA/RNA bases¹⁸⁻²⁰ and their homodimers²¹ have merged recently and have been made possible by the coupling of an electrospray source with a cold ion trap.²² Well-resolved vibronic spectroscopy can thus be recorded and assigned through comparison with *ab initio* quantum mechanics calculations. Studying model systems gives insight into the photochemistry of protonated nitrogen heterocycles that in turn aids in understanding biologically relevant photochemistry. The simplest protonated aromatic molecule, benzene, and aromatic nitrogen heterocycle, pyridine, have been characterized at medium resolution only very recently.^{23,24} The electronic spectrum of protonated benzene is structure-less, which is in agreement with the large geometrical changes and the fast dynamic toward internal conversion predicted by *ab-initio* calculations.²⁵ Concerning protonated pyridine, the spectra recorded in the photon energy range 37000 – 45000 cm⁻¹ reveal a broad electronic band exhibiting vibronic resolved structure. The excited state structure is buckled in a prefulvenic geometry. This

behavior is a familiar feature of many small aromatics²⁶⁻²⁸ and is also observed for neutral pyridine.²⁹⁻³² Very recently, Broquier et al. have undertaken the investigation of protonated ortho, meta and para aminopyridine molecules^{33,34} and have shown that although protonated aminopyridines are rather simple aromatic molecules, their deactivation mechanisms are indeed quite complex. In the present study, the spectroscopy of the protonated 2-, 3- and 4-hydroxypyridine (HPH⁺) is investigated across the photon energy range 33 500 – 41 000 cm⁻¹ using an experimental device coupling an electrospray ion source with a cold quadrupolar ion trap and a time of flight mass spectrometer. We present the vibrationally resolved electronic spectra of cold HPH⁺ along with dissociation kinetics through one- and two-color photofragmentation spectroscopy. Ultraviolet-ultraviolet hole-burning (UV-UV HB) spectroscopy was also used to discriminate conformational isomers in the case of the protonated meta 3-hydroxypyridine.

2 – Experimental and computational methods

2-, 3- and 4-hydroxypyridine were purchased from Sigma Aldrich and directly dissolved in water/methanol mixtures (50/50 by volume) at 100 μ M and together with few droplets of acetic acid. The electronic spectra were obtained via photo-fragmentation spectroscopy in a cryogenic cold quadrupole ion trap QIT (Paul trap from Jordan TOF Products, Inc.) installed at the CLUPS facility in Orsay. The setup is similar to the one developed by X. B. Wang *et al.*³⁵ and has already been described elsewhere.^{36,37} The protonated species are produced from an Electrospray Ion Source (ESI). They pass through a 10 Hz pulsed octopole ion guide that produces ion packets with a duration time between 500 ns and 1 μ s, and are then trapped in the QIT which is mounted on a cold head of a compressed helium cryostat that maintains the temperature around 10-15 K. A mass gate located before the entrance of the trap allows for mass-selecting the parent ion. The photodissociation laser is triggered after few tens of millisecond when thermalization of the ions through collisions with helium buffer gas is reached, and all ionic fragments and parent molecules are then extracted and accelerated for mass-analysis in a linear time-of-flight mass spectrometer. The photodissociation laser is either a picosecond Optical Parametric Amplifier (OPA), with a spectral resolution of 8 cm⁻¹, or a nanosecond dye laser (Quantel TDL 90), which resolution is about 0.2 cm⁻¹.

For the fragmentation kinetics measurements, the fragmentation time is recorded by changing the delay between the laser and the extraction pulse ejecting the ions from the ion trap to the time-of-flight. The time resolution, on the order of 80 ns, is limited by the rise time of the high voltage pulser.

UV-UV hole burning (HB) spectroscopy (using two dye lasers) has been performed in order to reveal the possible distinct isomers that are present in the trap after the low temperature cooling process. We have used the tickle method initially proposed by Kang *et al.*,³⁸ which consists of applying on one cap of the QIT an auxiliary RF whose frequency is tuned to selectively eject a given ionic fragment. By doing that, the chosen ionic fragment of a first UV laser (burn), set on a given vibronic band, is ejected from the trap, which allows recording the signal induced by a second UV laser (probe) scan, over the whole spectral range, without background signal. The tickle RF voltage is kept below 1 V and sent for 10 ms after the excitation from the burn laser. In order to increase the HB signal-to-noise ratio, we run the burn laser at 5 Hz while keeping the probe at 10 Hz and perform the experiment using the modified active baseline subtraction methods (ABS).^{39,40}

Computational methods

Ab initio calculations have been performed with the TURBOMOLE program package (v6.2)⁴¹ making use of the resolution-of-the-identity (RI) approximation for the evaluation of the electron-repulsion integrals.⁴² The equilibrium geometries of protonated 2-, 3- and 4-hydroxypyridines in their ground electronic (S_0) and excited states have been determined at the CC2 level with the correlation-consistent polarized valence double- ζ aug-cc-pVDZ basis set augmented with diffuse functions.⁴³ The vibrational modes of the ground and the first excited states have been calculated at the same level, and the Franck-Condon analysis has been performed using PGOPHER software.⁴⁴

Isomers

Hydroxypyridines are protonated at the pyridinic nitrogen and can adopt two conformations according to the position of the oxygen lone pair as compared to the position of the proton. The conformer with the lone pair oriented towards the proton will be denoted *syn*, and the conformer with a rotation of π around the C-O bond will be denoted *anti*. For protonated 2-hydroxypyridine, the *anti* conformer is likely to be unstable due to the repulsion between the proton bound to nitrogen and the hydroxyl hydrogen. For the protonated 4-hydroxypyridine, the two isomers are identical due to the NCO symmetry axis. Only in the case of protonated 3-hydroxypyridine, the two isomers can coexist and are energetically close. As compared to the neutral molecule, the protonated hydroxypyridine can hardly display tautomerization because the keto form would correspond to a positively charge doubly hydrogenated nitrogen atom with a sp^3 tetrahedral molecular orbital structure calculated 1.7 eV higher than the initial sp^2 hybridization.

3 – Results

Protonated 2-Hydroxypyridine : 2-HPH⁺

Figure 1a shows the vibrationally-resolved UV photofragmentation spectrum obtained with the ps OPA laser on a large spectral domain, from 34 500 cm⁻¹ up to 2000 cm⁻¹ above. The UV excitation of 2-HPH⁺ (m/z 96) results in the dissociation into m/z 78 ions, which corresponds to the loss of H₂O. This fragment, which is also the main dissociation channel in CID, is produced immediately with a kinetics of formation shorter than the time resolution of the experiment defined by the rising time of 80 ns of the extraction pulse from the QIT.

The vibronic spectrum of 2-HPH⁺ is composed of intense and sharp bands, the first one being observed at 35 435 ± 5 cm⁻¹, while low intensity transitions are barely seen in the red of this intense band. The higher resolution spectrum obtained with the dye laser is reported in Fig. 1b and confirms that the first weak vibronic band is indeed recorded at 34 806 ± 5 cm⁻¹, i.e. about - 630 cm⁻¹ from the intense band. These weak transitions cannot be assigned to hot bands because they are too far in the red from the intense band. In the low frequency region, there is a vibrational progression of +82 cm⁻¹ built from the first transition at 34 806 cm⁻¹ with at least 2 quanta with increasing intensity. This suggests a geometry distortion in the excited state along a low frequency mode, leading to a weak band origin. The low signal makes a firm assignment of all the observed transitions difficult. We can nevertheless recover the same vibrational progression built from a band at 390 cm⁻¹ above the first transition. In overall, the vibronic spectrum of 2-HPH⁺ indeed closely resembles to the one recorded for protonated 2-aminopyridine.³³ As it will be discussed below, these two sets of intense and weak transitions will be assigned to two distinct minima in the excited state of 2-HPH⁺, as observed for protonated 2-aminopyridine. The band origin of 2-HPH⁺ is red-shifted by more than 1300 cm⁻¹ as compared to neutral 2-hydroxypyridine.^{7,9-10}

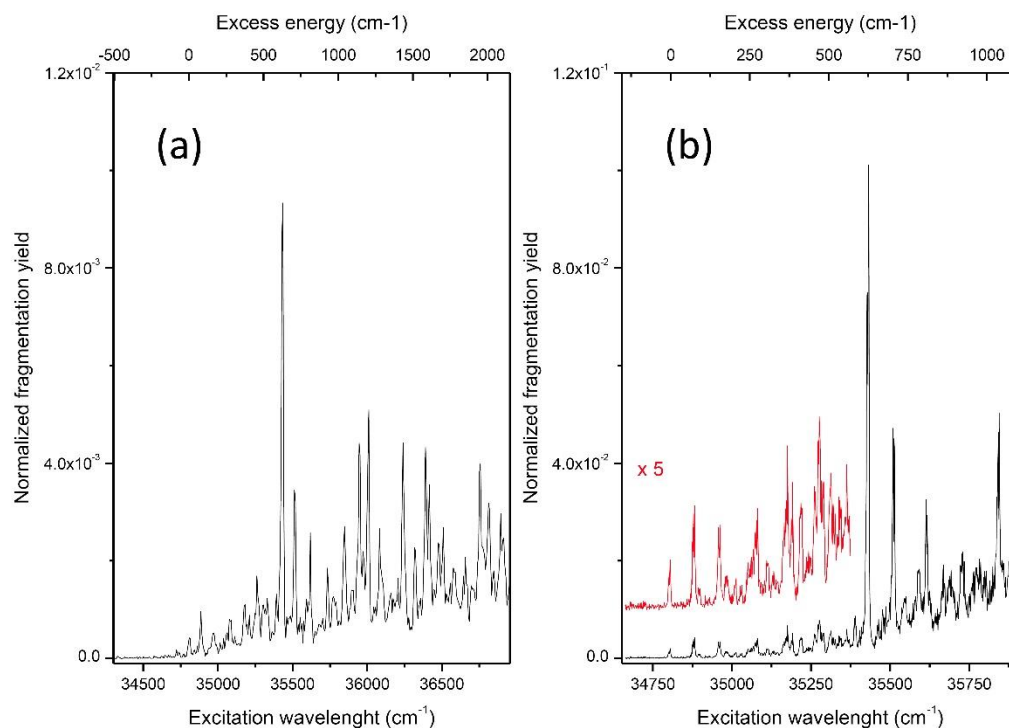


Figure 1. Photofragmentation spectrum of protonated 2-HPH⁺ recorded on the m/z 78 ionic fragment. (a) on the left, the spectrum is obtained over a large spectral range with the ps OPA laser (8 cm⁻¹ resolution). (b) on the right, low frequency region obtained with the dye laser (0.2 cm⁻¹ resolution).

Protonated 3-Hydroxypyridine : 3-HPH⁺

The vibrationally resolved vibronic spectrum of 3-HPH⁺, shown in Figure 2a, is obtained with the OPA laser from 33 500 cm⁻¹ up to 3000 cm⁻¹ above. The UV-induced fragmentation produces mainly two fragment ions that are also observed through CID: m/z 68, which is the main fragment and corresponds to the loss of CO, and m/z 78, which is ten times less intense and corresponds to the loss of H₂O, as observed for 2-HPH⁺. A fragmentation kinetics of 210 μs for the two fragmentation channels has been measured and is consistent with a dissociation in the ground state following internal conversion. As for 2-HPH⁺, the vibronic spectrum is composed of a set of intense transitions starting at 34 357 ± 5 cm⁻¹ along with low intensity bands further in the red. The higher resolution spectrum recorded with the dye laser (Fig. 2b) allows asserting the position of the first weak transition at 33 814 cm⁻¹, *i.e.* about 540 cm⁻¹ in the red of the intense band. Here again, these weak bands are too red-shifted to be assigned to progression from hot bands and the overall vibronic spectrum closely resembles the one recorded for 2-HPH⁺ with two sets of intense and weak vibronic transitions.

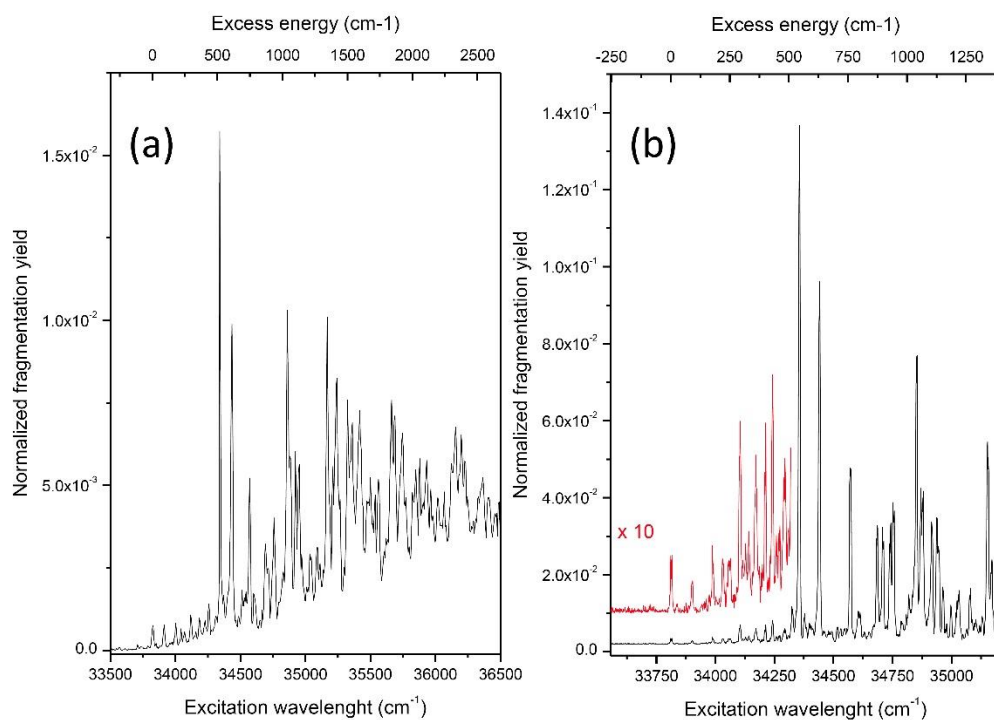


Figure 2. Photofragmentation spectrum of protonated 3-HPH⁺ recorded on the m/z 68 ionic fragment. (a) on the left, the spectrum is obtained over a large spectral range with the ps OPA laser (8 cm^{-1} resolution). (b) on the right, low frequency region obtained with the dye laser (0.2 cm^{-1} resolution).

For 3-HPH⁺, two low-lying energy isomers (*syn* and *anti*) can be populated at low temperature. Ultraviolet-ultraviolet hole-burning spectroscopy was used to discriminate these conformational isomers. The very low intensity of the vibronic spectrum in the red of the first intense transition precludes to record any UV-UV HB signal in that spectral region. We thus turn to the region where intense transitions are detected. The burn laser was first set on the band at $34\,357 \text{ cm}^{-1}$ (Fig. 3a). Most of the vibronic transitions are detected in the HB spectrum except a first one at $34\,686 \text{ cm}^{-1}$. A second HB spectrum (Fig. 3b) is thus obtained by fixing the burn laser at this frequency. The two HB spectra allow recovering the entire vibronic spectrum of 3-HPH⁺, which clearly reveals that only two conformers contribute to the experimental spectrum, at least in that spectral region. It should be noted that the burn laser seems to induce some hole-filling signals as reflected by few and weak positive HB bands for the two burn wavelengths.

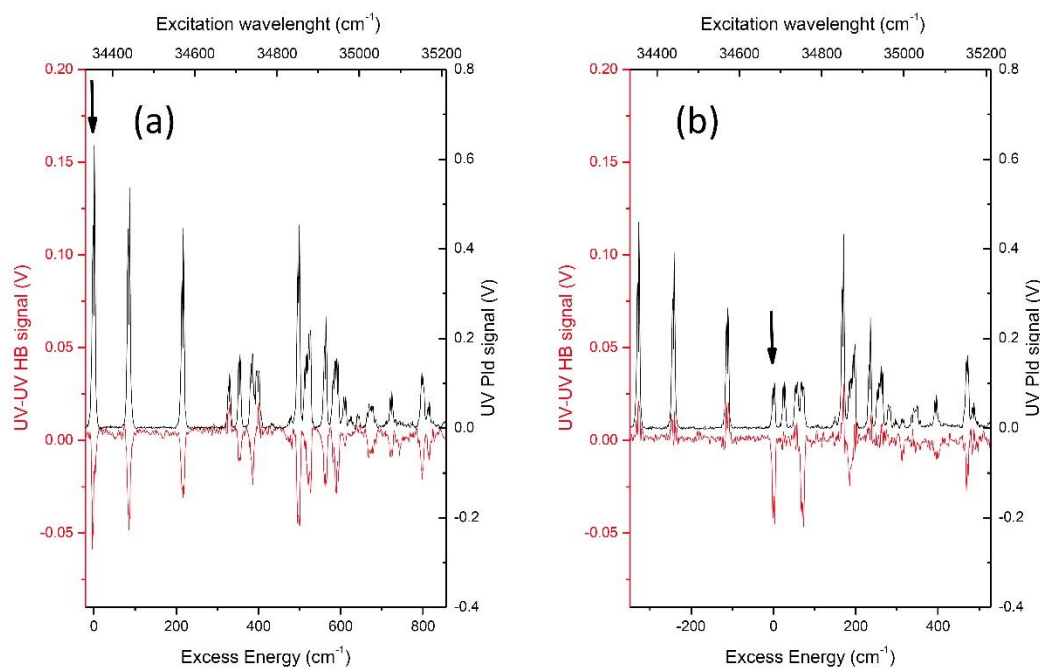


Figure 3. UV-UV hole-burning spectra of 3-HPH⁺ obtained at two burn laser frequencies (indicated by an arrow) of (a) 34 357 cm⁻¹ and (b) 34 686 cm⁻¹. For each plot (same vertical scale), the top trace (black line) is the UV photo-induced (PID) signal while the bottom trace (red line) is the UV-UV hole-burning (HB) signal.

Protonated 4-Hydroxypyridine : 4-HPH⁺

In that case, the fragmentation leads to three fragments: the main fragment is m/z 68 (CO loss) along with m/z 78 (H₂O loss) and a weak signal at m/z 69 (HCN loss). The fragmentation kinetics is in the order of 2.5 ms which is much longer than for 2- and 3-HPH⁺. As for the other molecules, such long fragmentation kinetics strongly suggests that dissociation occurs in the ground electronic state after internal conversion. The photofragmentation spectrum of 4-HPH⁺ recorded with the ps OPA laser (Fig. 4a) is quite noisy and much less structured than the ones for 2- and 3-HPH⁺. With the dye laser, only a couple of sharp transitions are observed before an intense one followed by the onset of a broad continuum starting from roughly 40 350 cm⁻¹. The first transition is located at 39 660 cm⁻¹ and should be assigned to the band origin. This 0₀⁰ transition is significantly blue shifted as compared to those of protonated 2- and 3- hydroxypyridines. A first vibrational band is found 120 cm⁻¹ above the origin transition while the most intense band appears 637 cm⁻¹ higher in energy. As for the two other isomers, an intense vibronic transition is detected about 600 cm⁻¹ above the first weak transition of the excitation spectrum but the overall vibronic spectrum is clearly different.

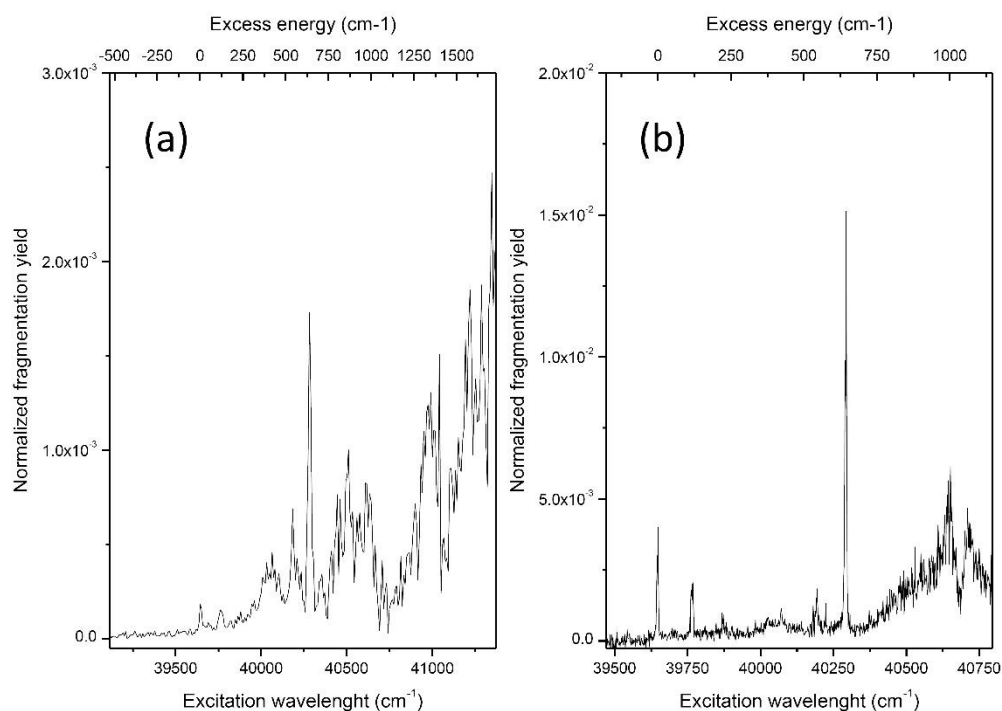


Figure 4. Photofragmentation spectrum of protonated 4-HPH⁺ averaged on the three ionic fragments at $m/z = 78, 69$ and 68 . (a) on the left, the spectrum is obtained over a large spectral range with the ps OPA laser (8 cm^{-1} resolution). (b) on the right, low frequency region obtained with the dye laser (0.2 cm^{-1} resolution).

4 - Discussion

Vertical and adiabatic excited state calculations

Ab initio coupled-cluster (CC2 / aug-cc-pVDZ) calculations have been performed for the three HPH⁺ molecules. As expected, the *syn* conformer is more stable than the *anti* conformer by 4.15 kcal/mol and 0.95 kcal/mol for the 2- and 3-HPH⁺, respectively. While the former one is thus not expected to be populated at low temperature, 3-HPH⁺ should adopt both conformations. Since the calculated electronic properties are similar and share the same trends for the *syn* and *anti* structures of 3-HPH⁺, we will discuss only the results of the *syn* conformer. For all molecules, the ground state equilibrium structure is planar, all HPH⁺ molecules thus belong to the C_s point group. The vertical excited state energies of 2- 3- and 4-HPH⁺ are reported in Table 1 along with the corresponding frontier molecular orbital representation. The first low lying excited states are all of $\pi\pi^*$ character and no $n_N\pi^*$ is present since the lone pair of the pyridinic nitrogen is involved in bonding with the proton. The $\pi\sigma_{NH}^*$ state is calculated more than 2 eV above the $\pi\pi^*$ state, which bears the oscillator strength for UV excitation, so that it is not involved in the deactivation process in HPH⁺. In 4-HPH⁺, the first and second $\pi\pi^*$ states are almost degenerated and strongly mixed, with several orbitals involved in the frontier MO decomposition of the states. In these planar ground state structures,

the lone pair of the hydroxyl group (p_z orbital) is in the same plane as the π orbitals of the pyridine ring and contributes to the $\pi\pi^*$ transition.

Table 1. Electronic configurations and vertical transition energies (eV) of 2- 3- and 4-HPH⁺ (CC2/aug-cc-pVDZ) *syn* conformers. In bold, $\pi\pi^*$ transition bearing the oscillator strength for UV excitation.

	2-HPH ⁺ (C_s)		3-HPH ⁺ (C_s)		4-HPH ⁺ (C_s)	
	Configuration	Energy	Configuration	Energy	Configuration	Energy
S ₁	$\pi\pi^*$ (A') (4a''-5a'')	4.82	$\pi\pi^*$ (A') (4a''-5a'')	4.59	$\pi\pi^*$ (A') (3a''-6a'') + (4a''-5a'')	5.43
S ₂	$\pi\pi^*$ (A') (4a''-6a'')	6.27	$\pi\pi^*$ (A') (4a''-6a'')	5.75	$\pi\pi^*$ (A') (3a''-5a'')	5.58
S ₃	$\pi\pi^*$ (A') (3a''-5a'')	7.19	$\pi\pi^*$ (A') (3a''-5a'')	6.72	$\pi\pi^*$ (A') (4a''-5a'') + (3a''-6a'')	6.59
S ₄	$\pi\sigma_{NH}^*$ (A'') (4a''-27a')	7.28	$\pi\sigma_{NH}^*$ (A'') (4a''-22a')	7.07	$\pi\pi^*$ (A') (4a''-6a'')	7.39

Geometry optimizations of the first $\pi\pi^*$ state have been performed for the three HPH⁺ molecules without symmetry constraint. In all cases, the optimization has converged to a planar $\pi\pi^*$ state preserving the C_s symmetry. However, these C_s structures are saddle points of the potential energy surface (PES) with one imaginary frequency of roughly -50/-80 cm^{-1} depending on the molecules. The associated mode corresponds to out-of-plane butterfly motion of the pyridinic ring. With such a low negative frequency in the $\pi\pi^*$ state, we cannot assert from the calculations whether or not the C_s structure is a saddle point or a true secondary minimum of the $\pi\pi^*$ PES.

In order to escape from the planar C_s geometry in S₁, the structures have been distorted along the calculated negative frequency mode. For 2- and 3-HPH⁺, this has led to slightly non planar geometries in a boat-like, prefulvenic structure, the N₁H and C₄H groups pointing above the aromatic ring (See Fig. 5). These optimized structures are true minima of the PES without imaginary frequency. These two adiabatic structures closely resemble the N-pyridinium excited state structure calculated at the DFT/CAM-B3LYP/aug-cc-pVDZ by Hansen et al.²⁴, although the nitrogen pyramidalization is less pronounced in the present case. In pyridinium, the barrier height to planarity is calculated at 2000 cm^{-1} , while in 2- and 3-HPH⁺, CC2/aug-cc-pVDZ calculations predict a very small barrier of about 100 cm^{-1} (see Table 2).

Such small energy difference, which more or less matches the amplitude of the negative frequency of the C_s structure, is certainly within the error of the CC2 method and the relative energy of the two structures in the excited state should be taken with great care. Experimentally, for 2- and 3-HPH⁺, the first intense transition is blue-shifted by about 600 cm^{-1} as compared to the weak band

origin. As it will be discussed below, the Franck-Condon analysis allows deciphering the contribution of two minima of the $\pi\pi^*$ PES in the experimental vibronic spectra of 2- and 3-HPH⁺.

For 4-HPH⁺, geometry optimization following distortion along the butterfly mode has led to a conical intersection with the ground state 0.3 eV below the energy of the Cs structure. At this point, the pyramidalization at the protonated nitrogen is large with the adjacent CH group pointing almost perpendicular to the pyridine cycle. The access, through a very small barrier, from the Franck-Condon region (Cs structure) to the conical intersection in 4-HPH⁺ is consistent with the absence of a vibrational progression in the vibronic spectrum of 4-HPH⁺.

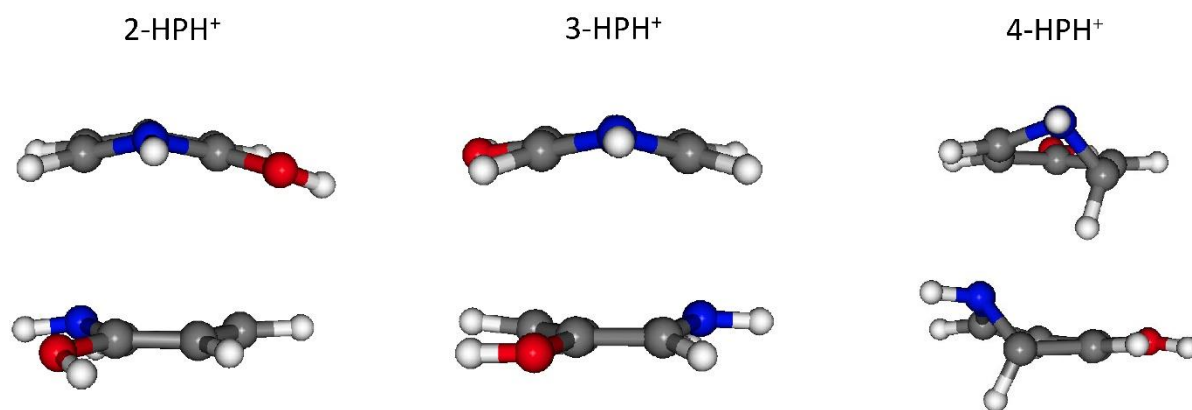


Figure 5. $\pi\pi^*$ excited state optimized prefulvenic structures of 2- and 3-HPH⁺. For 4-HPH⁺, the geometry optimization leads to a conical intersection with the ground state, point where the optimization is stopped.

Table 2. Experimental and calculated $\pi\pi^*$ energy transition of 2-, 3- and 4-HPH⁺. Calculated energy transitions (CC2/aug-cc-pVDZ) corrected by the difference in zero point energy (ZPE) between the ground and excited states. All values are in cm⁻¹.

molecules	Symmetry	0 ₀ ⁰ calc	0 ₀ ⁰ exp	$\Delta(\text{calc-exp})$
2-HPH ⁺ <i>Syn</i>	prefulvenic	35 427	34 806	621
	Cs	35 522	35 435	86
3-HPH ⁺ <i>Syn</i>	prefulvenic	34 059	33 814	245
	Cs	34 124	34357	- 233
3-HPH ⁺ <i>Anti</i>	prefulvenic	34 337	-	
	Cs	34 481	34686	- 204
4-HPH ⁺	prefulvenic	IC	39 660	
	Cs	40 523	40 297	226

Franck-Condon analysis

The electronic spectrum of 4-HPH⁺ will not be discussed in this section because only a very few active vibronic bands are observed which precludes the assignment of the structure in the $\pi\pi^*$ PES. The vibronic spectra of 2- and 3-HPH⁺ exhibit two sets of sharp transitions with quite different intensities. The excited state calculations have revealed the presence of two close lying structures in the $\pi\pi^*$ PES, an adiabatic prefulvenic structure and a second one of planar Cs geometry as in the electronic ground state. This is exactly what was found in protonated 2-aminopyridine³³. Its electronic spectrum has been assigned to the combination of two vibronic progressions of these two PES minima that have rather different Franck-Condon (FC) activities. Intense transitions were calculated for the Cs structure that correspond to the band origin (BO) along with the in-plane mode (a') of the pyridinic ring, while the red-shifted low intensity bands are assigned to the adiabatic minimum with progression of out-of-plane modes. In 2- and 3-HPH⁺, the simulated vibronic spectra of the adiabatic minima have been calculated and their overall intensity are ten-fold weaker than those for the Cs structures. For 3-HPH⁺, the presence of the two conformers and the impossibility to perform the hole-burning spectroscopy in the low frequency region makes the assignment highly hypothetical. Comparison between the calculated and experimental spectra are thus reported in Fig. 6 for 2-HPH⁺. The calculated band origin has a very weak intensity. A progression of the butterfly mode ν_1 (a'') calculated at 90 cm⁻¹ built from the BO with a maximum at 3-4 quanta closely matches the experimental low frequency progression of 82 cm⁻¹ experimentally observed for 2-HPH⁺. The same ν_1 progression built from the Franck-Condon active modes ν_4 , ν_5 , ν_7 , ν_{13} and ν_{15} is calculated. If the intensities of the calculated 0_0^0 and ν_1 progression are scaled to the corresponding experimental bands, it is clear from these simulations that the intense experimental transitions from 630 cm⁻¹ above the BO cannot be attributed to the adiabatic minimum.

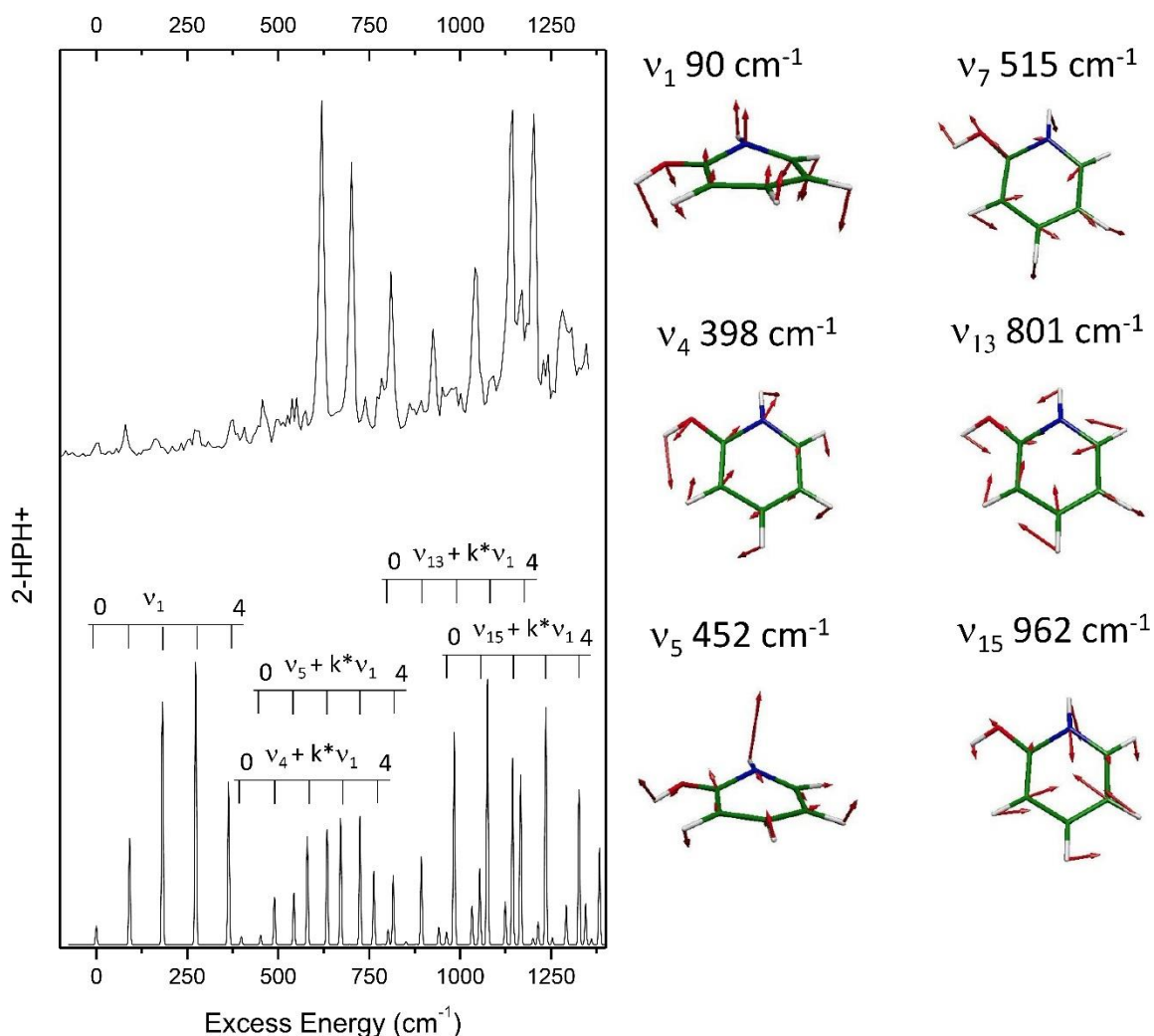


Figure 6. Comparison of the experimental (top) and Franck-Condon simulated spectrum of the prefulvenic structure of 2-HPH⁺.

In Figure 7, the experimental vibronic spectrum of 2-HPH⁺ is compared to the Franck-Condon simulations of the Cs structure (Fig. 7a) with its origin set at the most intense vibronic band (35 435 cm⁻¹) of the experimental spectrum. It is noteworthy that the calculated and experimental band origins (Table 2) of the Cs structure only differ by less than 100 cm⁻¹. Besides, their calculated transition intensities are one order of magnitude larger than the ones of the prefulvenic structure. Selection rules of the Cs point group impose that only totally symmetric a' vibrational modes are active along with even quanta of out-of-plane a'' modes. This is exactly reproduced in the simulated FC spectrum. The in-plane active modes of the Cs structure are calculated at 402, 503, 547, 804 and 958 cm⁻¹ and correspond to COH bending, 6a-b, mode 1 and 12b according to the Wilson notation, respectively (see Fig. 7). Only the first a'' mode (v₁ = 162 cm⁻¹) with two quanta has non negligible intensity in the calculated FC spectrum. While these active modes nicely match some of the intense experimental vibronic transitions, others are obviously missing in this simulation, in particular, the

two first transitions found at +76 and + 183 cm^{-1} from the 35 435 cm^{-1} band. The absence of the active low frequency modes of the Cs structure suggests that the missing bands might come from vibronic coupling between the prefulvenic and Cs minima.

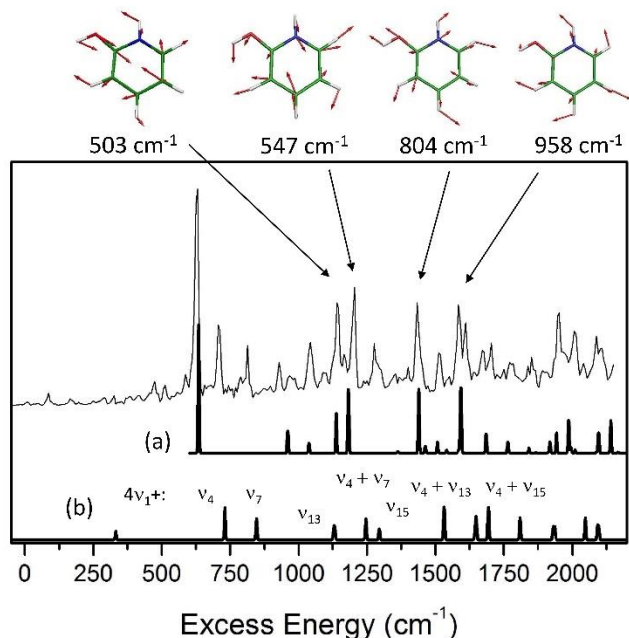


Figure 7. Comparison of the 2-HPH⁺ experimental (top) and calculated Franck-Condon spectra of (a) Cs structure and (b) the prefulvenic structure obtained as a combination of 4 quanta of the butterfly mode ν_1 with the in-plane (a') modes. All spectra are plotted as a function of excess energy (cm^{-1}) from the band origin of the prefulvenic structure. The in-plane (a') modes are reported for the Cs structure.

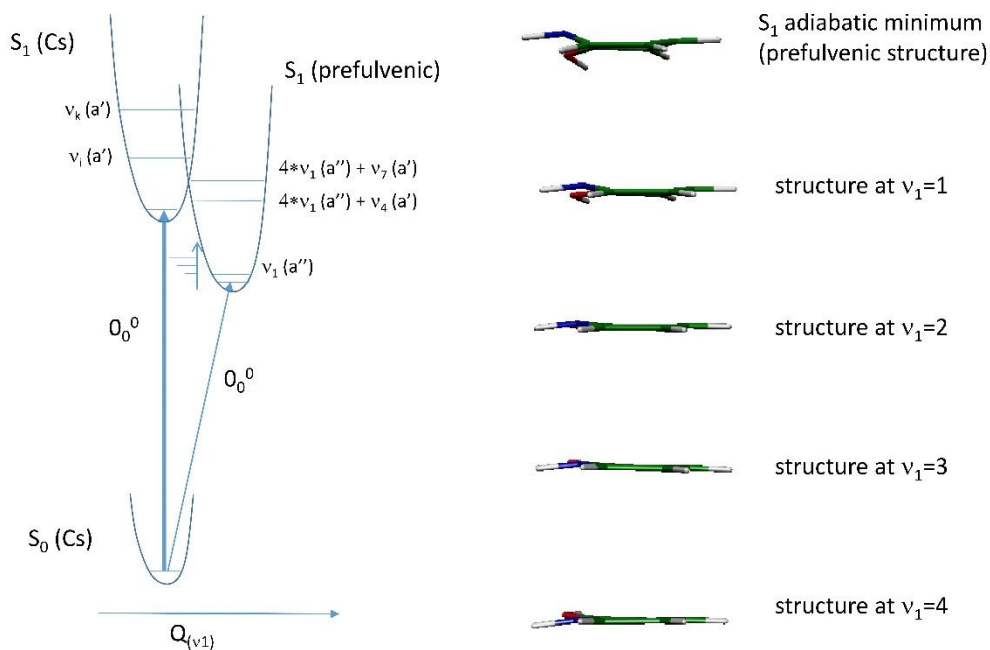


Figure 8. (left) Schematic diabatic representation of the double-well potential with $Q_{(\nu_1)}$ as the butterfly bending mode coordinate that connects the planar (Cs) and prefulvenic minima in S_1 . (Right) distorted structures of 2-HPH⁺ as a function of butterfly mode ν_1 progression.

Figure 8 shows a schematic diabatic representation of the double-well potential (harmonic approximation) with $Q_{(v_1)}$ as the butterfly bending mode coordinate relevant for this spectroscopic study. Vibronic transitions from the Cs ground state structure to the Cs excited state structure have large intensities for the in-plane (a') modes, while Franck-Condon active transitions to the prefulvenic excited state structure involve a progression of the butterfly v_1 mode. We have used the screw module in Turbomole to distort the 2-HPH⁺ molecule along the normal mode v_1 as a function of the temperature. Within the harmonic oscillator approximation, the energy of a vibrational normal mode i (E_i) is given by $E_i = 1/2 \omega_i^2 * Q_i^2$, where ω_i and Q_i are the wavenumber and normal coordinate of mode i , respectively. If one substitutes E by $k_b T$, (k_b for Boltzmann constant and T the temperature), the coordinates can be distorted along the butterfly mode ($v_1 = 90 \text{ cm}^{-1}$ for 2-HPH⁺) with a temperature step of 125 K. In the right panel of Fig. 8, the corresponding distorted structures of 2-HPH⁺ are reported, and it can be readily checked that with 3 or 4 quanta in v_1 , the prefulvenic structure has almost recovered the planarity. Indeed, the calculated FC spectrum of the prefulvenic structure (Fig. 6) reveals that the largest transitions are those with 3 quanta in v_1 , which scales the prefulvenic to planar coordinate displacement in the excited state. Qualitatively, a vibronic coupling between the prefulvenic and planar (Cs) minima should involve vibrations of the prefulvenic state that recover the planar symmetry (butterfly mode) and match the general selection rules of the Cs point group, *i.e.* totally symmetric a' modes. The simulated FC spectrum of Fig. 7b has been calculated as combination bands of 4 quanta in the butterfly mode and all the in-plane bending and stretching modes (a') of the adiabatic structure. Most of the missing transitions in the simulated spectrum of the Cs structure, in particular the two intense transition at $+ 76 \text{ cm}^{-1}$ and $+ 183 \text{ cm}^{-1}$ from the BO of the Cs structure, can now be assigned to the contribution of combination bands of the prefulvenic structure with in-plane (a') modes and 4 quanta of the butterfly mode needed to recover the planarity. This diabatic harmonic formalism used to simulate the experimental spectrum is certainly a crude approximation of the true adiabatic potential energy surface of the excited state. Nevertheless, it provides a very good qualitative and satisfying quantitative agreement with the experimental spectrum.

The same procedure has been applied to 3-HPH⁺ to assign the intense vibronic bands of the experimental spectrum. As already pointed out, two conformers account for all the intense vibronic transitions. The band origins of the *syn* and *anti* conformers in the planar Cs structure have been assigned through the hole-burning spectroscopy and compared to the calculated ones. The *syn*

conformer has its BO at 34 357 cm⁻¹, 329 cm⁻¹ to the red of the BO of the *anti* conformer. Both the absolute and relative adiabatic transition energies are very well reproduced by the calculation, in particular the splitting between the two conformers predicted at 357 cm⁻¹, *i. e.* within 30 cm⁻¹ of the experimental one (see Table 2). The UV-UV hole-burning spectrum of the *anti* conformer (Fig. 3b) covers a limited spectral range of 500 cm⁻¹ where only the first two in-plane active modes (a') of the Cs structure are detected at 388 and 500 cm⁻¹ along with combination bands involving 4 quanta of the mode ν_1 of the adiabatic minimum. In the Cs structure, the frequencies of in plane modes (breathing vibration of the cycle) are not quite different for the three HPH⁺, even less for the *syn/anti* conformers of 3HPH⁺. Nevertheless, as for 2-HPH⁺, the intense vibronic bands assigned to the *syn* conformer in Figure 3a can be reproduced with the active in-plane a' modes of the Cs minimum along with combination of 4 quanta of the butterfly motion with in-plane a' modes of the prefulvenic minimum structure (Fig. SI1).

5 – Conclusions

We have investigated the photofragmentation processes in protonated hydroxypyridine through a combined experimental and theoretical study. Although protonated ortho, meta and para hydroxypyridine appear as simple model molecules that mimic the molecular subunits comprising larger biologically relevant compounds, the understanding of their vibronic spectroscopy is more complex than expected. These three molecules show well resolved electronic excitation spectra, with a strong influence of the position of the hydroxyl group. In particular, the band origin of 4-HPH⁺ is significantly blue-shifted in comparison with 2- and 3-HPH⁺. UV-UV hole burning spectroscopy for the 3-HPH⁺ clearly reveals the presence of two *syn*- and *anti*- conformers at low temperature. For all molecules, the weak intensity of the band origin along with the progression on low frequency modes reveals a geometry change in the excited state, as predicted by the prefulvenic structure found through excited-state optimized geometry calculated at the CC2 / aug-cc-pVDZ. Besides, the intense vibronic transitions are assigned to the planar Cs structure that corresponds to tenfold larger intensities. For both adiabatic and Cs structures, the calculated band origins including ZPE corrections are in good agreement with the experimental data within an absolute error of about 500 cm⁻¹. Finally, a strong vibronic interaction between the prefulvenic and planar structures in the excited state seems to account for the experimental spectra.

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Supplementary Materials:

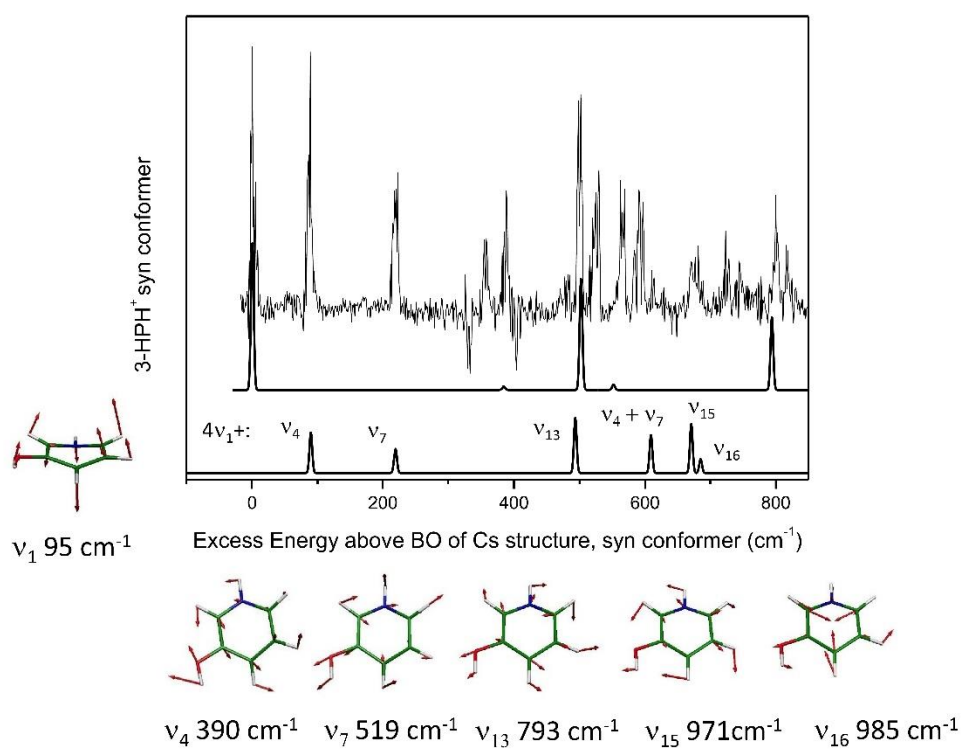


Figure S11: Comparison of the experimental (top) and Franck-Condon simulated spectra of the 3-HPH⁺ *syn* conformer.