

Structure and dynamics in Formamide-(H₂O)₃ adduct: a water-pentamer analogue

Susana Blanco*, Pablo Pinacho and Juan Carlos López*

Departamento de Química Física y Química Inorgánica, Facultad de Ciencias, Universidad de Valladolid, E-47011 Valladolid, Spain.

ABSTRACT: Water self-association dominates the formation of microsolvated molecular clusters which may give rise to complex structures resembling those of pure water clusters. We present a rotational study of the complex formamide-(H₂O)₃ formed in a supersonic jet and several monosubstituted isotopologues. Formamide and water molecules form a four body sequential cycle through N-H...O, O-H...O and O-H...O=C hydrogen bonds, resulting in a chiral structure with a non-planar skeleton that can be overlapped to that of water pentamer. The analysis of the ¹⁴N-nucleus quadrupole coupling effects shows the depletion of the electron density of the N atom lone pair with respect to the bare formamide that affects to the amide group C-N and C=O distances. The study of the observed tunneling doublets shows that formamide-(H₂O)₃ follows a path to invert its structure driven by the flipping of water subunits and passing through successive non-planar configurations, a motion reminiscent of the pseudorotation of water pentamer.

Keywords: hydrogen bonding, microsolvation, rotational spectroscopy, water dynamics, nanodroplets

The investigation of the initial steps of the solvation process through the study of microsolvated molecular systems has been an important subject for Chemistry.^{1,2,3} The corresponding molecular clusters can be isolated in the cold environment of supersonic jets and spectroscopically probed. Rotational resolved spectroscopy techniques are ideal for this purpose since they have an inherent high resolution and are exceptional tools to determine the structure.^{4,5,6} Rotational studies of microsolvated model organic molecules^{7,8,9,10,11,12,13,14,15,16,17} have provided a better understanding on the solvent interactions in large biomolecules,¹⁸ the role of hydrogen bond (HB) cooperativity¹⁹ and on the way in which solvation induces structural changes in the solute molecule.^{12,17,20} This research has also led to better understanding the association processes showing the interplay between self-association and solvation. In fact, with few exceptions,²⁰ one of the most important observations in complexes with several H₂O molecules is that water prefers to link other water molecules forming chains or cycles. In clusters with only one HB acceptor site^{10,13,14} the structures of the complexes reflect a trade-off between maximizing the number of water-solute interactions, and the minimum-energy

structures of the pure water clusters. In molecules with double HB donor/acceptor character, water molecules close sequential cycles¹⁹ as observed in amides^{7,8,9} acids,^{15,16} or esters.¹¹ However with the exception of some organic acids,^{15,16} there is no information on clusters with more than two water molecules on the last kind of systems.

The complexes of formamide, the smallest system having an amide group, with water (formamide-(H₂O)_n, n=1,2)^{7,8} see Figure 1) have been used as a benchmark to model the local interactions of water in proteins. Their structures could suggest that formamide-(H₂O)_n clusters would resemble (H₂O)_{n+2} clusters so that formamide might fit in the structure of high order water clusters.^{4,5,6,21,22} However, this hypothesis could be demonstrated from the microsolvated clusters of formamide with n>2. In this context we present the rotational study of the cluster formamide-(H₂O)₃ (f-w₃). One of the main objectives was to compare its structure and dynamics with those of the chiral structure of water pentamer. A second purpose was the observation of the structural signatures of cooperative HB, especially the characterization of inductive polarization effects related to resonance assisted hydrogen bonding (RAHB)¹⁹ scarcely observed in the gas phase^{9,17} through the ¹⁴N nuclear quadrupole coupling constants which have been shown¹⁷ to be an experimental probe alternative to the elusive detection of small C-N or C=O structural changes.

A theoretical investigation²³ on the possible forms of f-w₃ (see Supporting Information) predict the lowest energy forms as those in which f-w₃ adopts a cyclic arrangement, being f-w₃-c1 (Figure 1) the global minimum. The spectrum of a species identifiable as one of the cyclic forms was observed to show the hyperfine structure characteristic of species having one ¹⁴N nuclei plus an additional splitting attributable to a tunneling motion (Figure 2). It was analyzed²⁴ using a two state Hamiltonian including semirigid rotor,²⁵ quadrupole coupling²⁶ and Coriolis coupling terms.²⁷ A summary of the results is given in Table 1. The spectra of the ¹⁵NH₂-CHO···(H₂O)₃, NH₂-¹³CHO···(H₂O)₃, the three NH₂-CHO···(H₂¹⁸O···(H₂O)₂) isotopologues and the monodeuterated species of the atoms involved in the HB were also recorded. The details of the analysis, the complete results and the frequency tables are given in the Supporting Information.

A first insight on the structure can be obtained from the analysis of the determined planar moment of inertia P_{cc} (7.03654(6) uÅ²) which measures the mass extension out of the *ab* inertial plane. It can be compared with the values for related species like formamide (-0.0036 uÅ²),²⁸ the planar skeleton complexes formamide_m-(H₂O)_n (m,n=1,2)^{7,8,17} (0.054-0.6541 uÅ²), or the value of ca. 1.4 uÅ² estimated for a planar skeleton of f-w₃ with three out-of-plane water hydrogen atoms. This allows us to

conclude that the skeleton of the f-w₃ complex is not planar. The changes in the planar moments upon isotopic substitution indicate that all heavy substituted atoms are out of the *ab* inertial plane.

We have determined the r_s^{29} and r_0^{30} structures from the rotational constants of the parent species and isotopologues. The results, given in supplementary information, are summarized in Figure 3. The r_s method, a purely experimental approach, gives the absolute coordinates of each substituted atom in the principal axis system of the parent species (the signs can be taken from the r_0 or r_e structure). It allows to directly locate the different subunits of a complex although it poses limitations for atoms located near the principal axes. The r_0 method provides a total or partial determination of the effective ground state structural parameters from a least squares fit of all of the available rotational parameters.

The comparison of the r_s , r_0 and r_e structures indicates that the observed form corresponds to conformer f-w₃-c1 (Figure 3). The experimental structure reflects that formamide and water subunits close a sequential cycle through four HBs, C=O⋯H-O, O-H⋯O, O-H⋯O and O-H⋯N thanks to the HB donor/acceptor double character of both molecules. The HB lengths indicate that the dominant interactions are O-H⋯O. The shorter distance of the O-H⋯O=C compared to that of O-H⋯N indicates that the interaction of water with the carbonyl group is stronger than with the amino group. The effects of σ -bond cooperativity¹⁹ can be clearly seen from the comparison of the observed structure of f-w₃ with those of previously observed complexes of formamide and water^{7,8} (see Figure 1, Tables 2 and S20). This confirms that the H⋯O, O⋯O and N⋯O distances associated to HBs decrease as the number of water molecules increase, as a characteristic of sequential cycles.

The comparison of the average O⋯O distances and O⋯O⋯O or N⋯O⋯O angles of f-w₃ with those of water clusters (H₂O)_n with n=2-5 (see Table 2)^{21,22,31-33} indicate that these are close to those of water pentamer.^{21,22} In fact, the observed structure of the f-w₃ is so close to that of the predicted more stable form of w₅³⁴ that even the orientation of the non-bonded hydrogen atoms of the -w₃ fragment are practically the same. Both complexes are in fact superimposable (Figure 4). The geometry of w₅ is usually associated to that of a five membered-ring so it is reported to have a bent-ring configuration with a bending angle β of 164.5°.²² A similar configuration can be devised for f-w₃ so for the r_0 structure a bending angle of 157.3(5)° and a small twisting angle τ of -8.0(6)° are estimated. The similarities extend to other couples (Figure 4) like f/w₂, f-w-a/w₃ and f-w₂/w₄ which can be approximately superimposed. The origin of this behavior lies in the similar geometrical arrangement of the C=O and N-H groups in formamide with the groups having the same HB donor/acceptor roles in the water

dimer. In this way formamide could be able to substitute the position of a water dimer fragment in water clusters so the structure of f-w_n complexes might turn out to be similar to (w)_{n+2} clusters.

Resonance assisted hydrogen bonding (RAHB), often called π -cooperative bonding,¹⁹ was first pointed out to occur in the case of the peptide linkage.^{35,36} Its main effect is the simultaneous peptide C=O bond enlarging and C-N bond shortening when amide dimers are formed. This effect has been experimentally observed in gas phase for f₂-w¹⁷ and from the structure determined in this work, it seems to take place also for f-w₃ (see Scheme 1). However, it was not observable for other formamide-water complexes^{7,8} and the structure comparison along the series should be based on theoretical predictions as shown in Figure S5. Nevertheless, this missed experimental information could be supplied by the analysis of the ¹⁴N quadrupole coupling effects. These arise from the interaction of the electric quadrupole moment, eQ, of the ¹⁴N nucleus with the electric field gradient *q* at the nitrogen nucleus site.²⁶ The associated spectroscopic constants $\chi_{\alpha\beta} = eQq_{\alpha\beta}$ ($\alpha, \beta = a, b, c$, the principal inertial axes) are very sensitive to changes in the electronic environment at this nucleus.

We have shown for formamide₂-water¹⁷ that the quadrupole coupling constant χ_{cc} reflects the changes due to RAHB inductive effects (Figure S5). In bare formamide, the *c* inertial axis, perpendicular to the plane of the molecule, is parallel to one of the principal ¹⁴N quadrupole coupling axis, say *z* ($\chi_{cc} \equiv \chi_{zz}$). Given the skeleton planarity of the formamide-water complexes studied so far^{7,8,17} it can be reasonably assumed that $\chi_{cc} \approx \chi_{zz}$. In f-w₃ the angle between the *c* inertial and the *z* quadrupole axes is of only 13° thus the χ_{zz} constant can be confidently estimated from the *ab initio* results. The ¹⁴N χ_{zz} constant can be related to the unbalanced $2p_z$ electronic charge $(U_p)_z = [(n_x - n_y)/2 - n_z]$ by:²⁶

$$\chi_{zz}/eQq_{210} = -(U_p)_z \quad (1)$$

where n_α are the p_α orbital occupation numbers and q_{210} is the electric field gradient associated to a $2p$ electron in an isolated atom ($eQq_{210} \approx -10$ MHz for N). In Figure 5, the experimental values of χ_{zz}/eQq_{210} for the different formamide-water clusters are plotted vs. $-(U_p)_z$ calculated from a natural bond orbital (NBO) analysis (see Figure S6).³⁷ Despite the approximations, they show an excellent correlation. According to the definition of $(U_p)_z$, the electron density excess along the *z* axis in formamide progressively decreases. This electron density can be associated to the population of the N-atom lone pair which is calculated to deplete correspondingly (Figure S7).

The calculated values of η_D (see Table S21) show noticeable changes from formamide and f-w-b, which are predicted to have an almost symmetric distribution of charge ($\eta_D = 0.04$), to formamide-H₂O-a ($\eta_D = 0.22$) and the three (f₂-w and f-w₂) and four (f-w₃) body clusters ($\eta_D = 0.35$ - 0.38 range). All the magnitudes considered through this discussion, the constant χ_{cc} , the bond lengths C-N and C=O or the η_D parameter seem to have similar values for the complexes of three and four bodies. This indicates that while adding a water molecule to the f-w-a complex to form f-w₂ notably alters χ_{cc} , C-N and C=O, or η_D , adding a new water molecule to form f-w₃ practically does not increase polarization effects so much. Probably this is a good reason to wonder to what extension the concept of RAHB could be related to cooperativity.³⁸

The doublets observed in the spectra indicate that the “puckered” configuration of f-w₃ has a two-fold degeneracy. If we consider the inversion through the planar C_s configuration of the adduct it is easy to see that both degenerate forms constitute a pair of specular images that cannot be superimposed, being thus enantiomeric forms. The observed structure of f-w₃ is chiral and the tunneling splitting of about 370 MHz ($0.0090(4)$ cm⁻¹) observed in the spectrum of f-w₃ arise by a vibrational motion connecting the two equivalent enantiomeric structures.

The analysis of the potential energy surface (PES) of f-w₃ to determine the nature of the observed splittings is not easy due to its multidimensional nature. The complete inversion of the structure requires motions associated to the reorientation of water molecules concerted to the “ring-puckering” motions of heavy atom skeleton. The water motions include the rotation around the HB axes (flipping) or the more energetic bifurcation mechanism.³² The inversion of the structure passing through the planar configuration by simultaneous torsion of all three water molecules require a rather high barrier, calculated in the range 700 cm⁻¹ to 950 cm⁻¹ (see Figure S8). Simple estimations with one-dimensional double minimum potential functions predict the observed inversion splittings corresponding to barriers close to 300 cm⁻¹. For this reason it is possible to reject this path.

Alternative paths have been investigated such as those driving conformational change by the flipping motion of one water molecule at a time (see Figure 6). This path passes by the different energy minima calculated for cyclic forms of f-w₃. They have been labeled as f-w₃-udu (specular image -dud), f-w₃-diu (-uid) and f-w₃-dui (-udi) from the orientation up (u), down (d) or in the plane (i) of the non-bonded hydrogen atoms of the three water molecules w₍₁₎, w₍₂₎ and w₍₃₎,

respectively. It can be seen that flipping of $w_{(1)}$, drives the change from the global minimum $f-w_3-udu$ to $f-w_3-diu$. Then $w_{(2)}$ rotation brings the system from $f-w_3-diu$ to $f-w_3-dui$ and finally $w_{(3)}$ flipping brings the cluster to $f-w_3-dud$ inverting the puckered form of the skeleton and completing the inversion of the structure from the starting point $f-w_3-udu$ to its enantiomeric form $f-w_3-dud$. Each motion drives a change in the conformation of the cluster through a reasonably small potential energy barrier. The non observation of $f-w_3-diu$ and $f-w_3-dui$ conformers confirms the low barrier since collisional relaxation might take place in the supersonic jet.³⁹ From this description at the origin of the observed vibrational doubling of the rotational spectrum it is easy to devise a periodic potential energy function inverting the structure without passing by the planar form. This periodic potential function would be similar to pseudorotation reported in five membered rings⁴⁰ and suggested for w_5 ²¹ and would be at the origin of the observed vibrational doubling of the rotational spectra of $f-w_3$.

In summary, the analysis of the spectrum of the complex of formamide with three water molecules has revealed interesting structural and dynamical features. The donor/acceptor hydrogen bonding properties of water and formamide lead to a structure in which the water molecules close a sequential cycle with formamide. In contrast with previously observed formamide-water clusters^{7,8,17} the heavy atom skeleton is not planar and adopts a chiral structure. One of the most interesting features of this structure is that it can be superimposed to the structure of the water pentamer. As a conclusion of this work we show that all the formamide- $(H_2O)_n$ clusters studied so far have structural similarities to $(H_2O)_{n+2}$ clusters. This is an interesting feature giving the total miscibility of formamide and water, which is observed even at the onset of microsolvation. The quadrupole coupling constants prove the progressive depletion of electronic density of the N-atom lone pair along a series of formamide-water complexes and that these constants are linearly correlated to the changes of the C-N and C=O distances associated to RAHB. These effects are noticeable in passing from the monomer to complexes with $n=1$ and 2, but are not so important in passing from $n=2$ to 3 water molecules. This led us to wonder to which extension RAHB ought to be considered within the context of cooperativity.³⁸ The small tunneling splitting results from the interconversion of the chiral observed structure into its specular image. The exploration of the potential energy surface suggests a mechanism driven by non-simultaneous flipping motions of the water molecules. This dynamics can be taken as reminiscent of the complex vibrational motions of water pentamer, which are related to the pseudorotation of a five-

membered ring, reinforcing the structure similarity between these systems. In addition, these results led one to wonder if similar effects could take place in larger systems as proteins where water motions can couple to protein motions which are very important to define their functions.

Further research on higher order complexes of formamide and water would be done to confirm these results.

EXPERIMENTAL

The microwave spectra (5-13 GHz) were recorded using a molecular-beam Fourier transform microwave spectrometer (MB-FTMW), described elsewhere.⁴¹ The commercial samples of formamide were heated at about 50°C, water was placed in a reservoir inserted in the gas line, and a He-Ne gas mixture was used as the carrier gas (backing pressure *ca.* 12 bar) dragging the molecules to form the complexes in the jet expansion. A detailed description of the experiment is included in the Supporting Information.

ASSOCIATED CONTENT

Details of the experimental conditions, description of the microwave spectra analysis, summary of the *ab initio* results, determined rotational parameters and measured experimental frequencies, complete structures compared to related systems, quadrupole coupling constants for different formamide water clusters.

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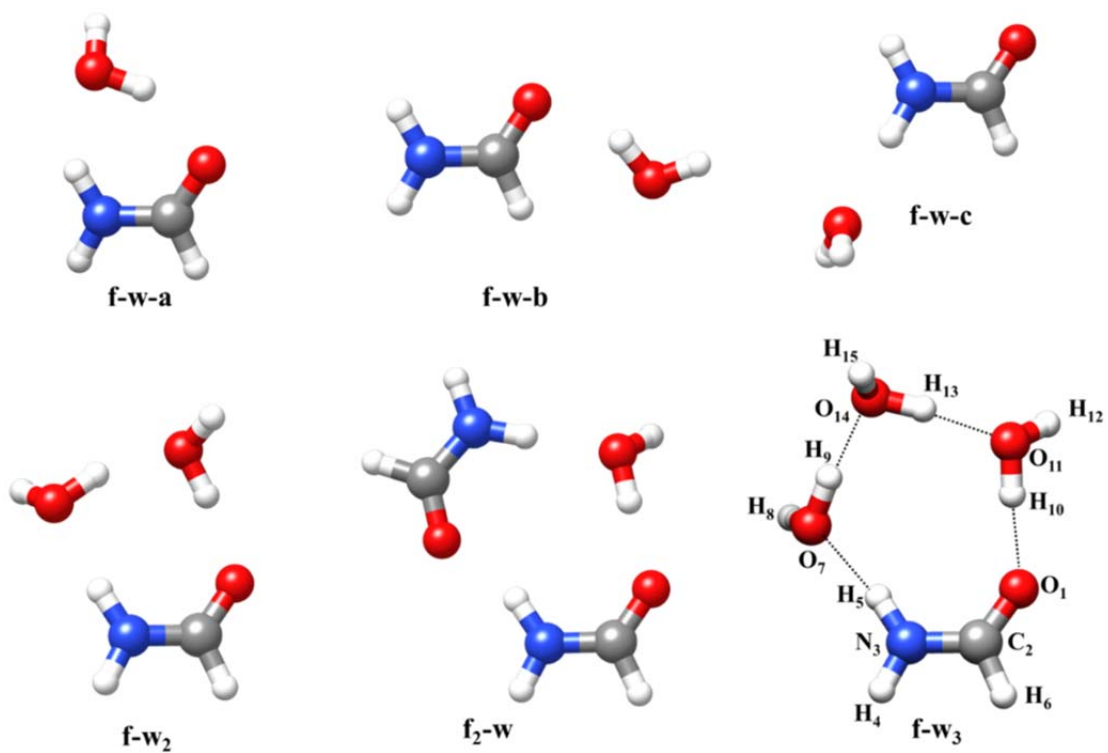


Figure 1. Observed formamide_m-(H₂O)_n (m=1,2; n=1-3) clusters and atom labeling for formamide-(H₂O)₃ (f-w₃).

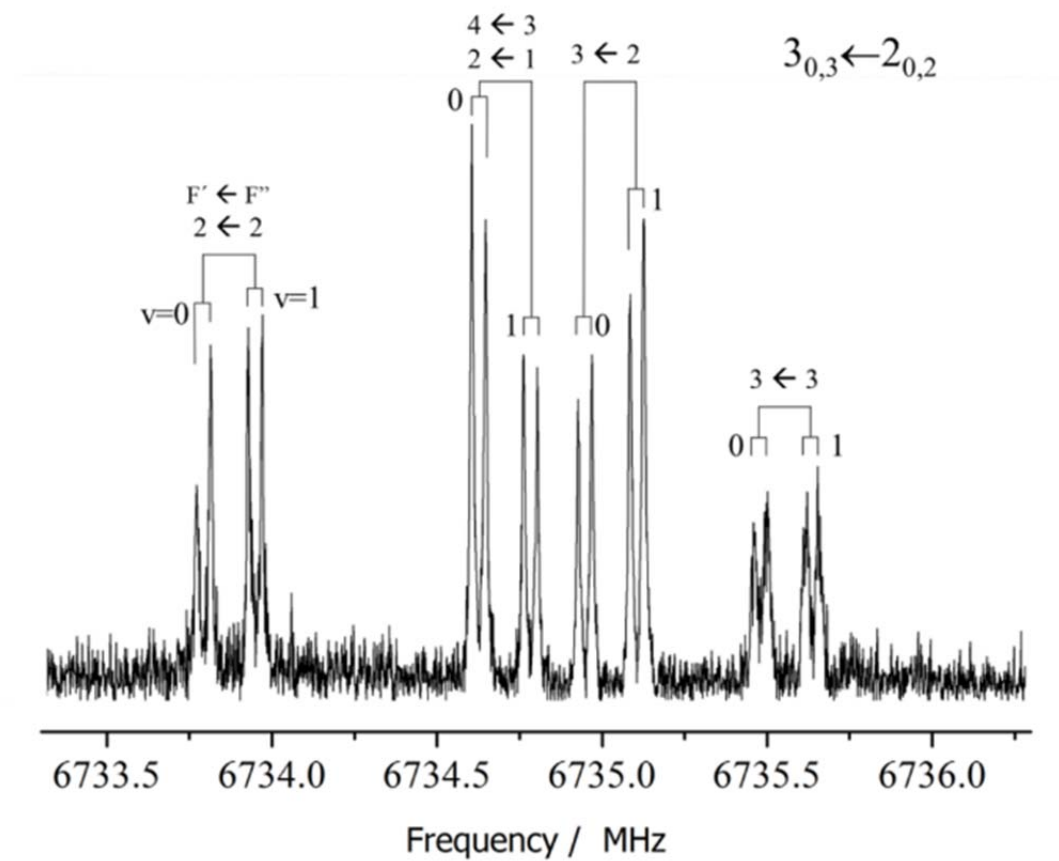


Figure 2. The $3_{0,3} \leftarrow 2_{0,2}$ rotational transition of the formamide-(H₂O)₃ (f-w₃) complex showing the quadrupole hyperfine components labeled with the F quantum number (F'←F"). The additional splitting in two levels labeled v=0 and v=1 is due to a tunneling vibration. Each line is further split due to Doppler effect.

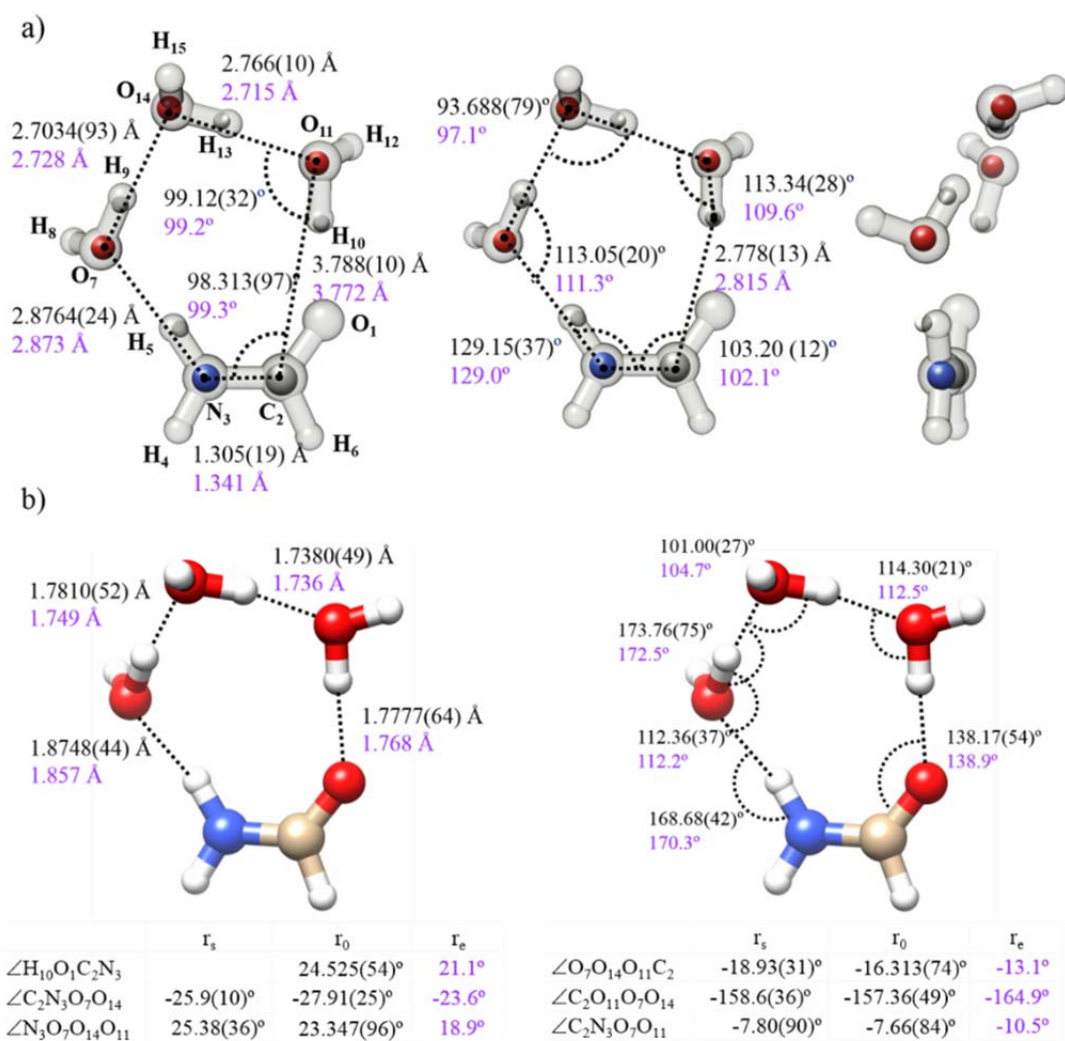


Figure 3. a) Comparison of the experimental r_s position of the isotopically substituted atoms of formamide-(H₂O)₃ (f-w₃) with the *ab initio* MP2/6-311++G(2d,p) structure. The r_s interatomic distances and angles are also given. b) The experimental r_0 bond lengths and angles of f-w₃ compared to the *ab initio* predictions.

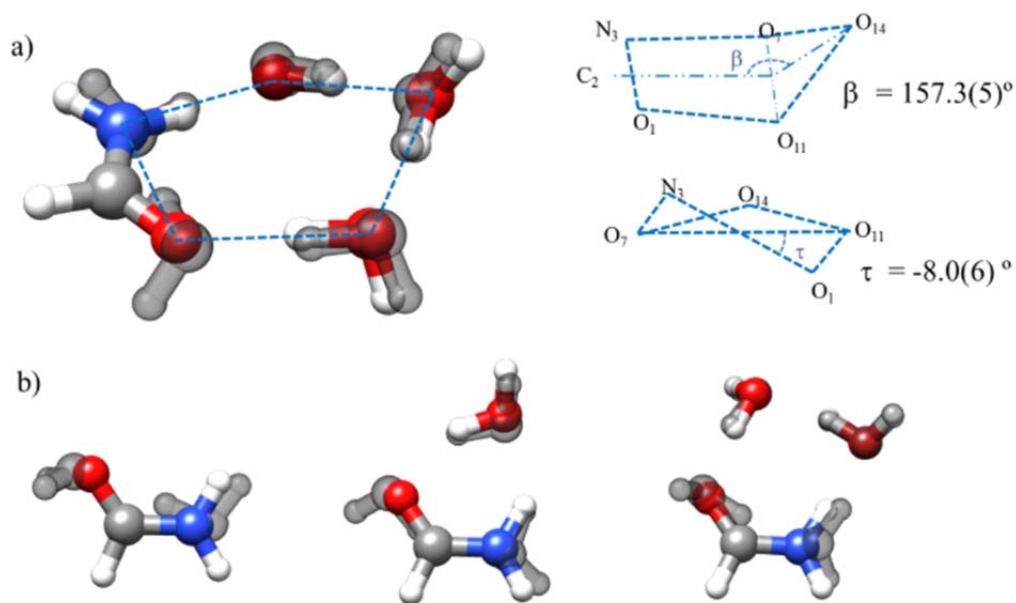


Figure 4. a) A comparison of the structure of formamide-(H₂O)₃ (f-w₃) found in this work with that of the global minimum of (H₂O)₅ (w₅) (superimposed in grey) and definition of the β and τ dihedral angles describing the conformation of a five-membered ring. b) The geometry similarities between formamide (f) and (H₂O)₂ (w₂), formamide-(H₂O)-a (f-w-a) and (H₂O)₃ (w₃) or formamide-(H₂O)₂ (f-w₂) and (H₂O)₄ (w₄) are shown (water clusters superimposed in grey).

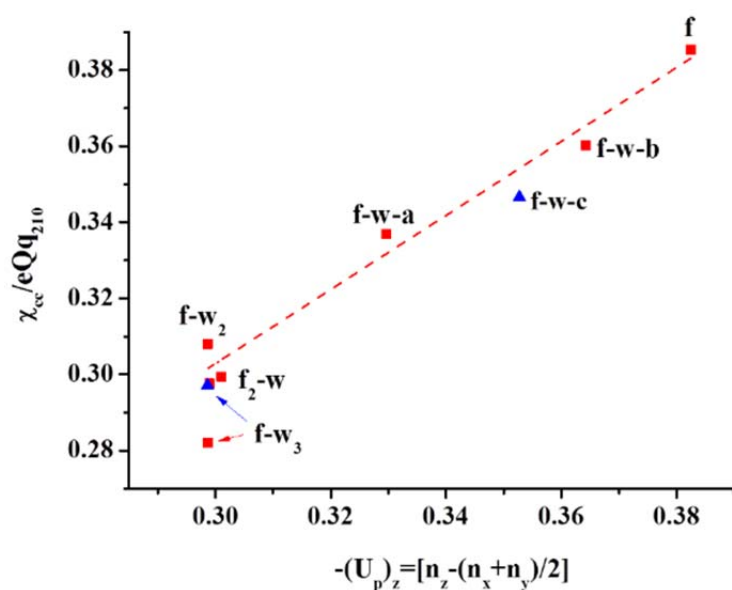


Figure 5. Plot showing the correlation between the experimental values (■) of the quantity χ_{cc}/eQq_{210} and the unbalanced $2p_z$ NBO electronic charge $-(U_p)_z$ (slope 0.97) for formamide (f), the formamide- H_2O forms (f-w-a and f-w-b), formamide- $(H_2O)_2$ (f-w₂), formamide₂- H_2O (f₂-w) and formamide- $(H_2O)_3$ (f-w₃) (see Figure 1). The points with triangle symbol (▲) correspond to scaled χ_{zz} *ab initio* values (see Table S21 and Figure S6) for formamide- H_2O form c (f-w-c) and f-w₃.

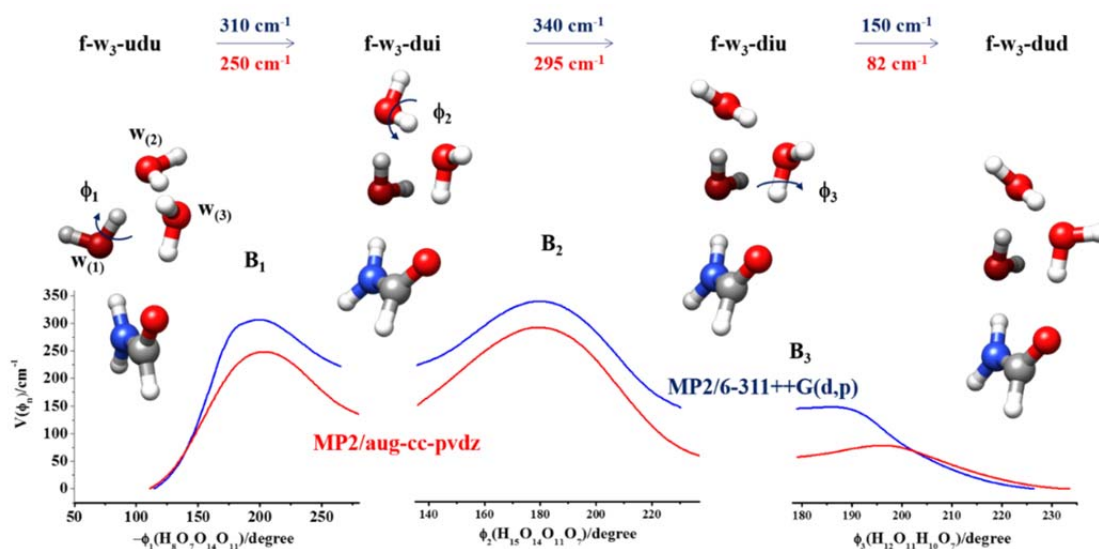
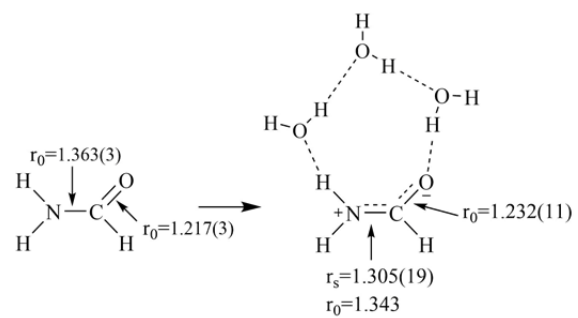


Figure 6. A possible path for inversion of the configuration of formamide-(H₂O)₃ (f-w₃) calculated in three steps by scanning successively the flipping angles ϕ_1 , ϕ_2 and ϕ_3 . Through this path, as in pseudorotation, the f-w₃ cluster transforms from the f-w₃-udu form to the f-w₃-dud enantiomeric equivalent forms passing through small barriers by the different stable conformers. Calculations have been done at MP2(FC)/6-311++G(d,p), MP2(FC)/6-311++G(2d,p) and MP2(FC)/aug-cc-pvdz levels. MP2(FC)/6-311++G(2d,p) give barriers intermediate between those shown in the figure.



Scheme 1. RAHB inductive effects in the formation of formamide-(H₂O)₃ (f-w₃) are evidenced by the C=O bond length enlargement and C-N bond length decrease.

Table 1. Rotational parameters obtained from the analysis of the spectrum of the observed formamide-(H₂O)₃ (f-w₃) cluster and calculated at the MP2/6-311++G(2d,p) level for the global minimum.

Parameters ^a	Exp. Value		f-w ₃
	v=0	v=1	
<i>A</i> /MHz	2552.02481(25) ^b	2552.02697(24)	2513
<i>B</i> /MHz	1458.29390(17)	1458.30123(17)	1497
<i>C</i> /MHz	952.624138(92)	52.656823(92)	957
<i>P</i> _{cc} /uÅ ²	7.03654(6)	7.04468(6)	5.4
χ_{aa} /MHz	0.8679(10)		0.86
χ_{bb} /MHz	1.9524(14)		2.02
χ_{cc} /MHz	-2.8204(14)		- 2.88
ΔE_{01} /MHz	370.4(47)		
$\langle 0 jG_a 1 \rangle$	1.1275(92)		
<i>n</i>	319		
σ /kHz	2.6		

^a *A*, *B* and *C* are the rotational constants. $P_{cc} = (I_a + I_b - I_c)/2 = (\sum_i m_i c_i^2)/2$) is the planar moment of inertia derived from the inertial moments I_α ($\alpha = a, b$ or c). χ_{aa} , χ_{bb} and χ_{cc} are the quadrupole coupling constants for ¹⁴N. ΔE_{01} is the energy difference between the vibrational states labeled v=0 and v=1. $\langle 0|jG_a|1 \rangle$ is the Coriolis coupling term connecting both vibrational states. *n* is the number of hyperfine quadrupole coupling components fitted. σ is the rms deviations of the fit. ^b Standard errors are given in parentheses in units of the last digit.

Table 2. (a) Geometrical parameters (distances in Å, angles in degree) of formamide-(H₂O) (f-w-a), formamide-(H₂O)₂ (f-w₂) and formamide-(H₂O)₃ (f-w₃) clusters. (b) Geometrical parameter of w_n (n=2-5) clusters.

(a)	f-w-a ^b	f-w ₂ ^b	f-w ₃ ^a	
<i>r</i> ₀ (O ₁ -O ₁₁)	2.8231(35)	2.7755(30)	2.7621(60)	
<i>r</i> ₀ (O ₁ -H ₁₀)	1.9304(98)	1.7969(83)	1.7777(64)	
<i>r</i> ₀ (O ₁₁ -O ₁₄)		2.755(3)	2.7167(55)	
<i>r</i> ₀ (H ₁₃ -O ₁₁)		1.829(4)	1.7380(49)	
<i>r</i> _s (O ₁₄ -O ₇)			2.7606(53)	
<i>r</i> ₀ (H ₉ -O ₁₄)			1.7810(52)	
<i>r</i> ₀ (O ₇ -N ₃)	2.8910(17)	2.9095(12)	2.8821(49)	
<i>r</i> ₀ (H ₅ -O ₇)	2.0607(45)	1.9363(60)	1.8748(44)	
∠(O ₁ -O ₁₁ -O ₁₄)			112.76(20)	
∠(O ₇ -O ₁₄ -O ₁₁)		86.30(15)	93.67(12)	
∠(N ₃ -O ₇ -O ₁₄)		84.04(13)	112.01(18)	

(b)	w ₂ ^c	w ₃ ^d	w ₄ ^e	w ₅ ^f
<i>r</i> (O-O)	2.976	2.85	2.78	2.76
∠(O-O-O)		60.	90.	107.9

^aThis work. ^bRef. 8. ^cRef. 31. ^dRef. 32. ^eRef. 33. ^fRefs. 21 and 22.