

## The complete conformational panorama of formanilide-water complexes: the role of water as conformational switch.

Pablo Pinacho, Susana Blanco\* and Juan Carlos López\*

Received 00th January 20xx,  
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

The microsolvated complexes of formanilide, generated in a supersonic expansion, have been observed by Fourier transform microwave spectroscopy. Three 1:1 and one 1:2 formanilide-water adducts have been observed and their structure characterized by the measurement of isotopologue rotational spectra. In one of the monohydrated complexes and in the dihydrated complex formanilide adopts a *cis*-configuration. In these species water closes sequential cycles with the *cis* amino and carbonyl groups through a network of N-H...O and O-H...O hydrogen bonds. Furthermore, in these complexes *cis*-formanilide has almost the same non planar configuration observed in the monomer. In the two monohydrated complexes detected with *trans*-formanilide, a planar skeleton is detected with water interacting solely with either the amino (N-H...O bond) or the carbonyl group (O-H...O=C bond). The observed tunnelling splittings show a rich intermolecular dynamics in those complexes. The results seem to indicate that complexation with water switches the configuration of formanilide from *trans*, more stable for the bare monomer, to *cis*, more stable for the hydrated complexes.

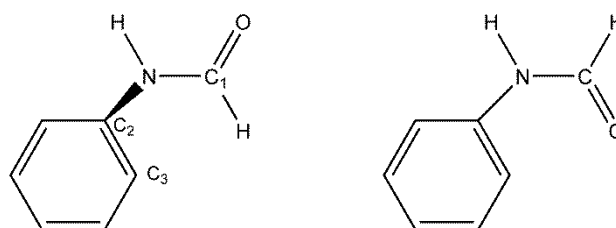
### 1 Introduction

The activity of biomolecules is closely related to their shapes. In solution, the forces driven by the solvent in part determine the shape. The interactions established between the solute molecules with water are mainly hydrogen bonds.<sup>1-3</sup> Water can drive changes in conformational<sup>4,5</sup> or tautomeric<sup>6,7</sup> equilibria, and induces the folding of proteins to their final active conformations.<sup>8,9</sup> The study of the interactions between water and biomolecules in condensed phases is a difficult task, but it is possible to characterize the forces established in the initial steps of the solvation process through the characterization of the complexes of model molecules with water in the gas phase. The environment in which a molecule interacts with a limited number of molecules of water is usually called microsolvation and it is a field of research with increasing relevance.<sup>10,11</sup> Microsolvated complexes with different degrees of hydration can be formed in supersonic jets and their structures characterized by rotationally resolved spectroscopic techniques. Fourier transform microwave spectroscopy (FTMW) coupled to supersonic expansion has proved to be an

excellent tool to determine the molecular structure of gas-phase complexes and to identify the non-covalent interactions involved in cluster formation.<sup>12-14</sup> Chirped-pulse FTMW spectroscopy is especially useful to identify the different complexes formed in the jet since it provides a direct snapshot of all the polar species present in the supersonic expansion. Rotational studies of microsolvated complexes of molecules bearing the amide group,<sup>5,15-19</sup> have served as a model to investigate how water establishes hydrogen bonds with the peptide linkage and the preferred sites for such interactions. In addition, these models have provided important pieces of information on cooperativity effects including  $\pi$ -cooperativity or resonance enhanced hydrogen bond affecting the structure of the amide group.<sup>17,18</sup> Formanilide (N-phenylformamide, C<sub>7</sub>H<sub>7</sub>NO, see Scheme 1) is a formamide derivative which presents a *cis-trans* conformational equilibrium.<sup>20-22</sup> In fact, a MB-FTMW study has shown that both forms of formanilide are present in the supersonic expansion being the *trans* conformer most stable by ca. 350 cm<sup>-1</sup>.<sup>22</sup> In this context, the observation of the microsolvated clusters of *cis*- and *trans*-formanilide would allow exploring the diverse interaction sites between

Dr. P. Pinacho, Prof. S. Blanco, Prof. J. C. López  
Departamento de Química Física y Química Inorgánica, Facultad de Ciencias,  
Universidad de Valladolid, E-47011 Valladolid, Spain  
E-mail: pablo.pinacho@uva.es, sblanco@qf.uva.es, jclopez@qf.uva.es

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x



Scheme 1. Formanilide in the *cis* (left) and *trans* (right) configurations. The angle between the amide and the phenyl ring in the *cis* configuration defined as the dihedral angle C<sub>1</sub>-N-C<sub>2</sub>-C<sub>3</sub> was found experimentally to be 34.7°.<sup>22</sup> The *trans* configuration is planar.

the peptide linkage and water and may give additional information on the water-driven plausible alteration of the *cis-trans* conformational equilibrium of formanilide.

The microsolvation of formanilide in a supersonic jet environment has been studied by laser-induced fluorescence excitation spectroscopy together with mass-selected, resonant two-photon ionization spectroscopy (R2PI), IR-UV ion dip and rotational band contour analysis,<sup>23–25</sup> binding energy measurements,<sup>26</sup> zero electron kinetic energy (ZEKE),<sup>27</sup> stimulated emission pumping (SEP),<sup>28</sup> and IR photodissociation (IRPD) spectroscopy.<sup>29</sup> In these works the experimental data attributed to two 1:1, three 1:2 and one 1:4 complexes between *trans*-formanilide and water are reported. Besides this, no experimental information on the *cis*-formanilide-water clusters was given, probably based on the assumption that the *cis* conformer of formanilide is less abundant in the supersonic jet.<sup>20–22</sup> In the present work, we report for the first time a high-resolution rotational study of the microsolvated complexes of both *cis* and *trans* formanilide.

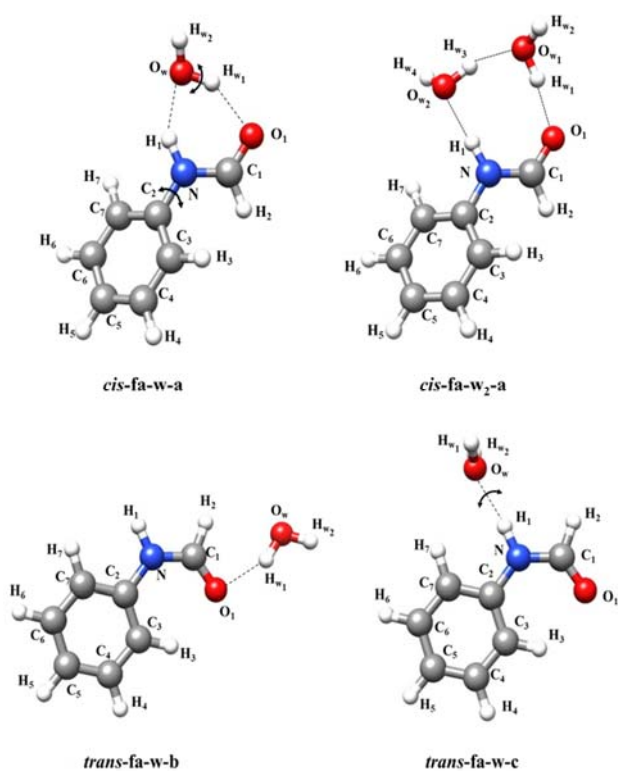


Figure 1. The complexes between formanilide and water observed in this work.

## 2 Experimental Section

Commercial samples of formanilide (m.p. 48°C), H<sub>2</sub><sup>18</sup>O and <sup>2</sup>H<sub>2</sub>O were used. The spectra of the formanilide-water complexes were investigated using a chirped-pulse Fourier transform microwave spectrometer (CP-FTMW), and a molecular-beam Fourier transform microwave spectrometer (MB-FTMW). In the CP-FTMW instrument, which follows Pate's<sup>30</sup> design and covers the 2–8 GHz frequency range, the

spectra were recorded in steps of 2 GHz. The carrier gas was Ar at backing pressures of about 3 bar expanding through a 1.0 mm nozzle in pulses of 900 μs duration. Chirp pulses of 4 μs were created by an arbitrary waveform generator and amplified to 20 W. The polarization signal was radiated from a horn antenna in a direction perpendicular to that of the expanding gas. A molecular transient emission spanning 40 μs is then detected through a second horn, recorded with a digital oscilloscope and Fourier-transformed to the frequency domain. The accuracy of frequency measurements is better than 10 kHz. In the MB-FTMW instrument,<sup>31,32</sup> operated in the 5–13 GHz frequency range, He-Ne mixtures were used at stagnation pressures ranging up to 4 bar expanding in pulses of about 800 μs through a 0.8 mm nozzle. Short (typ. 0.3 μs, 10–300 mW) microwave pulses were used for polarization purposes. Typically, a ca. 400 μs-length time domain spectrum was recorded in 40–100 ns intervals and converted to the frequency domain by a fast Fourier transform. Due to the collinear arrangement of the jet and resonator axis each rotational transition splits in two Doppler components so the resonant frequencies are taken as the arithmetic mean of both components. Frequency accuracy is better than 3 kHz. The rotational spectra of the H<sub>2</sub><sup>18</sup>O isotopologues were recorded in the MB-FTMW spectrometer. The spectra of the deuterated species were recorded in the CP-FTMW instrument.

Prior to start the experimental work, *ab initio* calculations<sup>33</sup> were performed to predict the rotational, centrifugal distortion, quadrupole coupling constants and electric dipole moment for the previously described formanilide-water complexes (see Figures S1–S2).<sup>23</sup> In this paper we have further explored the different configurations that water molecules may adopt in the complexes of *cis*-formanilide with water (see Figures S3–S4). Calculations were done at the B3LYP-D3/6-311++G(d,p), MP2/6-311++G(d,p), MP2/6-311++G(2d,p) and MP2/aug-cc-pvdz level of theory. Vibrational frequencies were obtained by considering only harmonic contributions. The rotational parameters calculated together with the predicted energies are collected in Tables S1–S4 in supplementary material.

## 3 Results and Discussion

### Microwave spectra.

The CP-FTMW spectrum was first analyzed after removing the known rotational transitions of *trans*- and *cis*-formanilide. Most of 1:1 and 1:2 complexes were predicted to be prolate asymmetric tops with the electric dipole moment mainly oriented along the *a* inertial axis. The corresponding <sup>a</sup>R-branch lines appear forming the characteristic J+1 ← J groups regularly spaced in frequency by B+C. Focusing the exploration of the spectrum on those patterns lead us to the assignment of the *cis*-formanilide...(H<sub>2</sub>O)-a (*cis*-fa-w-a), *cis*-formanilide...(H<sub>2</sub>O)<sub>2</sub>-a (*cis*-fa-w<sub>2</sub>-a), and *trans*-formanilide...(H<sub>2</sub>O)-b (*trans*-fa-w-b) species (see Figure 1). While the *cis*-formanilide complexes only show *a*-type spectra, the *trans*-fa-w-b species shows a weak *b*-type spectrum.

Table 1. Experimental rotational parameters for the *cis*-formanilide...( $\text{H}_2\text{O}$ )-a and *cis*-formanilide...( $\text{H}_2\text{O}$ )<sub>2</sub>-a complexes compared to the *ab initio* (MP2/6-311++G(2d,p) values.

Parameters <sup>a</sup>	<i>cis</i> -formanilide...( $\text{H}_2\text{O}$ )-a			<i>cis</i> -formanilide...( $\text{H}_2\text{O}$ ) <sub>2</sub> -a	
	Experimental		<i>ab initio</i>	Experimental	<i>ab initio</i>
	v=0	v=1			
A /MHz	2831.4048(56) <sup>b</sup>		2841	1992.9001(14) <sup>b</sup>	2007
B /MHz	662.38699(14)	662.38850(14)	664	494.434394(34)	496
C /MHz	549.51811(11)		554	405.007349(26)	409
$P_a / \text{u}\text{\AA}^2$	752.07627(21)		747.4	1008.18632(21)	1002.0
$P_b / \text{u}\text{\AA}^2$	167.60044(21)		164.2	239.64043(21)	234.9
$P_c / \text{u}\text{\AA}^2$	10.89011(21)		13.7	13.94930(21)	16.9
$\Delta_I / \text{kHz}$	0.04469(47)		0.04	0.058560(87)	0.05
$\Delta_{JK} / \text{kHz}$	[0.] <sup>c</sup>		0.02	-0.4858(11)	-0.37
$\Delta_K / \text{kHz}$	[0.]		1.78	[0.] <sup>c</sup>	2.18
$\delta_j / \text{kHz}$	0.01078(36)		0.01	0.016610(57)	0.02
$\delta_k / \text{kHz}$	0.480(27)		0.43	0.1667(46)	0.22
$3/2(\chi_{aa}) / \text{MHz}$	3.2149(93)		3.36	3.0130(72)	3.10
$1/4(\chi_{bb}-\chi_{cc}) / \text{MHz}$	0.9885(67)		0.89	0.9797(23)	0.91
$n$	246			156	
$\sigma / \text{kHz}$	2.5			0.8	

<sup>a</sup> A, B and C are the rotational constants.  $P_\alpha$  ( $\alpha = a, b$  or  $c$ ) the planar moments of inertia.  $\Delta_I$ ,  $\Delta_{JK}$ ,  $\Delta_K$ ,  $\delta_j$  and  $\delta_k$  the quartic centrifugal distortion constants,  $\chi_{aa}$ ,  $\chi_{bb}$  and  $\chi_{cc}$  the  $^{14}\text{N}$  quadrupole coupling constants.  $n$  is the number of components fitted.  $\sigma$  is the rms deviation. <sup>b</sup> Standard errors are given in parentheses in units of the last digit. <sup>c</sup> Parameters in square brackets were kept fixed.

Table 2. Experimental rotational parameters for the *trans*-formanilide...( $\text{H}_2\text{O}$ )-b and *trans*-formanilide...( $\text{H}_2\text{O}$ )-c complexes compared to *ab initio* (MP2/6-311++G(2d,p) values.

Parameters <sup>a</sup>	<i>trans</i> -formanilide...( $\text{H}_2\text{O}$ )-b			<i>trans</i> -formanilide...( $\text{H}_2\text{O}$ )-c	
	Experimental	<i>ab initio</i>	Experimental	<i>ab initio</i>	
			v=0	v=1	
A /MHz	4094.8678(75) <sup>b</sup>	4125	1608.09000(26) <sup>b</sup>	1607.55924(26)	1562
B /MHz	539.02116(10)	542	907.81842(11)	907.76796(11)	942
C /MHz	476.89366(10)	480	581.604742(46)	581.640188(46)	589
$P_a / \text{u}\text{\AA}^2$	936.94997(36)	931.4	555.68112(11)	555.61823(11)	535.2
$P_b / \text{u}\text{\AA}^2$	122.78105(36)	122.3	313.25781(11)	313.26775(11)	322.4
$P_c / \text{u}\text{\AA}^2$	0.63661(36)	0.3	1.01502(11)	1.10885(11)	1.2
$\Delta_I / \text{kHz}$	0.02959(23)	0.03		0.4732(12)	0.32
$\Delta_{JK} / \text{kHz}$	0.4586(77)	0.32		5.711(18)	3.04
$\Delta_K / \text{kHz}$	4.44(74)	1.75		-2.6208(51)	-1.52
$\delta_j / \text{kHz}$	0.00323(27)	0.003		0.21434(63)	0.14
$\delta_k / \text{kHz}$	[0.] <sup>c</sup>	0.23		0.1124(65)	0.07
$^{14}\text{N } 3/2(\chi_{aa}) / \text{MHz}$	2.802(16)	2.85		3.0600(42)	3.24
$^{14}\text{N } 1/4(\chi_{bb}-\chi_{cc}) / \text{MHz}$	1.3153(44)	1.33		1.16089(85)	1.19
$n$	111			274	
$\sigma / \text{kHz}$	2.5			1.3	

<sup>a</sup> A, B and C are the rotational constants.  $P_\alpha$  ( $\alpha = a, b$  or  $c$ ) the planar moments of inertia.  $\Delta_I$ ,  $\Delta_{JK}$ ,  $\Delta_K$ ,  $\delta_j$  and  $\delta_k$  the quartic centrifugal distortion constants,  $\chi_{aa}$ ,  $\chi_{bb}$  and  $\chi_{cc}$  the  $^{14}\text{N}$  quadrupole coupling constants.  $n$  is the number of components fitted.  $\sigma$  is the rms deviation. <sup>b</sup> Standard errors are given in parentheses in units of the last digit. <sup>c</sup> Parameters in square brackets were kept fixed.

It was observed that many other lines consisted of moderate intensity doublets that finally were identified as belonging to *a*- and *b*-type rotational transitions of the *trans*-formanilide $\cdots$ (H<sub>2</sub>O)-*c* species (*trans*-fa-w-c, see Figure 1). All the spectra show the hyperfine structure (hfs) due to the presence of a <sup>14</sup>N nucleus in the formanilide subunit. Once we had preliminary sets of rotational parameters we proceeded to measure the spectra with the molecular beam spectrometer (MB-FTMW) which has superior resolution and allows to completely resolve the quadrupole coupling hfs and any additional splitting (see Figures 2-3).

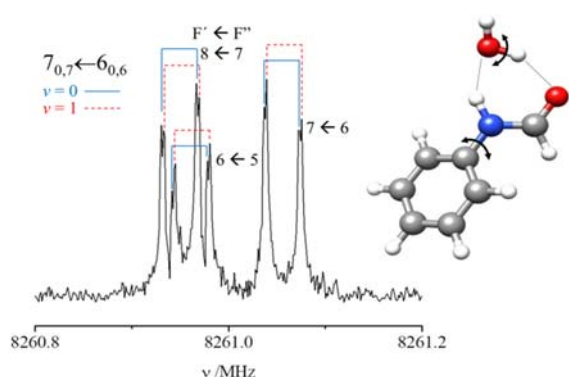


Figure 2. The  $7_{0,7} \leftarrow 6_{0,6}$  rotational transition for the *cis*-formanilide $\cdots$ (H<sub>2</sub>O)-*a* complex showing the <sup>14</sup>N quadrupole coupling hfs ( $F' \leftarrow F''$  transitions). The small splittings of *ca.* 2.5 kHz correspond to the rotational transitions in the  $\nu = 0$  (blue solid lines) and  $\nu = 1$  (red dashed lines) torsional states due to tunneling between two equivalent non-planar configurations. Each transition appears as a doublet because the Doppler effect.

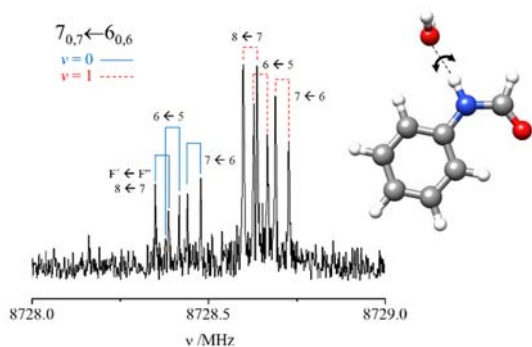


Figure 3. The  $7_{0,7} \leftarrow 6_{0,6}$  rotational transition for the *trans*-formanilide $\cdots$ (H<sub>2</sub>O)-*c* complex showing the <sup>14</sup>N quadrupole coupling hfs ( $F' \leftarrow F''$  transitions). The splitting of *ca.* 250 kHz, shows 1:3 statistical weight spin intensity effects characteristic of the internal rotation of water that exchanges the two hydrogen atoms. The assignment of the torsional states  $\nu = 0$  (blue solid lines) and  $\nu = 1$  (red dashed lines) was done on the basis of intensities. Each transition appears as a doublet because the Doppler effect.

While the spectra of *trans*-fa-w-b or *cis*-fa-w<sub>2</sub>-a do not show any splitting in addition to the <sup>14</sup>N hfs, the *cis*-fa-w-a spectrum shows small splittings of *ca.* 2.5 kHz attributable to rotational transitions in two vibrational levels very close in energy (see Figure 2). This splitting, only observable in the MB-FTMW spectrum, do not show statistical weight spin effects so as we

show below it can be in principle correlated to the vibrational motion that causes the doubling of bare *cis*-formanilide spectrum.<sup>22</sup> This is the torsion around the N-C<sub>2</sub> bond which interconverts two equivalent non-planar *cis*-formanilide structures through a small barrier at the planar configuration (see Figure 5 at Dynamics section). The torsional sub-levels of the complex were arbitrarily labeled as  $\nu = 0$  and  $\nu = 1$  for the lowest and highest frequency components respectively.

The *trans*-fa-w-c spectrum shows splittings of *ca.* 250 kHz (see Figure 3) together with the <sup>14</sup>N quadrupole coupling hfs. The components of the splittings present an alternation of 1:3 intensity ratio, which corresponds to the nuclear spin statistical weight effect of exchanging a pair of hydrogen atoms. The doublets were then attributed to the internal rotation of water molecule around the hydrogen bond axis, movement that exchanges the two hydrogen atoms from water. As a consequence each rotational transitions appears split into two torsional states, which could be assigned as  $\nu = 0$  and  $\nu = 1$  by taking into account the 1:3 intensity alternation. Apart from the parent species spectra those of all the monosubstituted H<sub>2</sub><sup>18</sup>O and some <sup>2</sup>H isotopologues were also measured. All spectra were analyzed employing Pickett's SPFIT program<sup>34</sup> using a Hamiltonian with semirigid rotor (A-reduction, I' representation)<sup>35</sup> and quadrupole coupling terms.<sup>36</sup> In the cases were vibrational doublets have been observed, a two-state Hamiltonian was used to constrain centrifugal distortion and quadrupole coupling parameters to have the same value in both states. The results for the parent species are compared to the *ab initio* predictions in Tables 1 and 2. The complete results are given in Tables S5-S8 and the observed experimental frequencies in Tables S15-S27.

### Structure

The degree of agreement between the rotational parameters obtained experimentally and those predicted (see Tables 1 and 2) suggests that the *ab initio* structures are a reasonable starting point to determine the structure of the different complexes by exploiting all the isotopic information.

The first point to consider concerning the structure of *cis*-fa forms is the value of the dihedral angle  $\angle C_1-N-C_2-C_3$ , reported to be 34.7° for the monomer.<sup>22</sup> The B3LYP-D3/6-311++G(d,p) level reproduces this value while MP2/6-311++G(d,p) or MP2/6-311++G(2d,p) predict values of this angle of around 40°. The experimental planar moment of inertia  $P_c$ , which gives the mass extension out of the *ab* inertial plane, increases significantly in passing from the *cis* monomer (3.9009 uÅ<sup>2</sup>)<sup>22</sup> to the *cis*-fa-w-a (10.8901 uÅ<sup>2</sup>), or to the *cis*-fa-w<sub>2</sub>-a (13.9493 uÅ<sup>2</sup>) (see Tables 1 and S9). This suggests that the structure of formanilide in both adducts is non planar. B3LYP-D3/6-311++G(d,p) calculations predict  $\angle C_1-N-C_2-C_3$  dihedral angle values close to 34.1° for both *cis*-fa-w-a and *cis*-fa-w<sub>2</sub>-a respectively, and 30.1° and 32.0° for the *cis*-fa-w-a' and *cis*-fa-w<sub>2</sub>-a' forms with different orientations of the non-bonded hydrogen atoms (see Figures S3-S4). The corresponding theoretical  $P_c$  values of 10.94 uÅ<sup>2</sup> and 11.85 uÅ<sup>2</sup> for conformers

*cis*-fa-w-a and *cis*-fa-w-a' respectively, or 13.37 uÅ<sup>2</sup> and 15.66 uÅ<sup>2</sup> for conformers *cis*-fa-w<sub>2</sub>-a and *cis*-fa-w<sub>2</sub>-a' (see Tables S1 and S3) led us to conclude that the observed forms are *cis*-fa-w-a and *cis*-fa-w<sub>2</sub>-a which on the other hand are predicted to be more stable. The experimental values of  $P_c$  for *cis*-fa-w-a and *cis*-fa-w<sub>2</sub>-a are reproduced at the MP2/6-311++G(2d,p) level by doing partial optimization of the structures by fixing the  $\angle C_1-N-C_2-C_3$  dihedral angle to 33.6° or 33.3°, respectively. These structures results in a good approach to the experimental rotational constants as shown in Table 1 (see also tables S5 and S8).

There are two methods to exploit isotopic data in order to obtain information on the molecular structure giving rise to the so called substitution,  $r_s$ , or effective,  $r_0$ , structures. The substitution method is based on the changes of the principal moments of inertia due to the substitution. It solves Kraitchman<sup>37</sup> equations to obtain the absolute values of the coordinates for the isotopically substituted atom in the principal inertial axis of the parent species. The signs of the coordinates can be taken from *ab initio* or  $r_0$  structures. Although this method has some drawbacks concerning light atoms or atoms close to inertial axis, it gives direct information on the structure which is crucial to the unambiguous identification of cluster structures. Alternatively a total or partial effective structure,  $r_0$ ,<sup>38</sup> can be obtained from a least-squares fit of all the available rotational constants.<sup>39</sup>

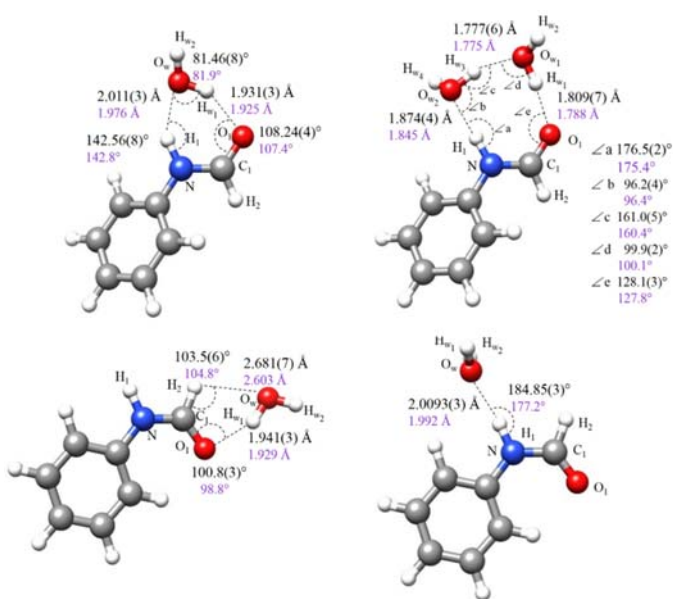


Figure 4. Experimental  $r_0$  hydrogen bond lengths and angles for the observed microsolvates of formanilide compared to the *ab initio* predictions.

The results of the application of both methods to the *cis*-fa-w-a and *cis*-fa-w<sub>2</sub>-a are given in Tables S10-S11. The uncertainties quoted for the  $r_s$  data were calculated according to the Costain rule.<sup>40</sup> The  $r_0$  structures are summarized in Figure 4. In the  $r_0$  fit the non-determined parameters were fixed to the *ab initio* MP2/6-311++G(2d,p) calculated values for the structure with  $\angle C_1-N-C_2-C_3$  fixed to reproduce the experimental  $P_c$  data. The experimental  $r_0$  value for this angle is of 33.5(1)° and 32.73(9)°

for *cis*-fa-w-a and *cis*-fa-w<sub>2</sub>-a, respectively, slightly lower than the angle 34.7° for bare *cis*-formanilide. The experimental value of the dihedral angle  $\angle C_1-N-C_2-C_3$  seems to steadily decrease from the monomer to the 1:1 and 1:2 hydrates in steps of *ca.* 1°. This behavior is in good agreement with theoretical calculations at different levels, which predict very small changes values for the equilibrium dihedral angle for the *cis* monomer and its complexes.

A second test to compare the structures of *cis*-formanilide and their microsolvated complexes can be done by subtracting the contribution of water to the inertial moments. This can be done from the  $r_s$  coordinates of water atoms,<sup>19,41</sup> which have been determined for *cis*-fa-w-a species. The resulting “dried” *cis*-formanilide  $A$  rotational constant has a considerable error, while  $B = 929.95(39)$  MHz and  $C = 793.73(27)$  MHz constants have values close to the average ones for *cis*-formanilide  $B = 929.2$  MHz and  $C = 794.7$  MHz.<sup>22</sup> This indicates that the structure of *cis*-formanilide does not experiment abrupt changes upon complexation.

The *trans*-fa-w-b and *trans*-fa-w-c complexes are expected to have a planar skeleton inheriting the planar structure of the *trans*-formanilide monomer. The experimental values for the  $P_c$  planar moments of inertia 0.6366 uÅ<sup>2</sup> and 1.0150 uÅ<sup>2</sup> for *trans*-fa-w-b and *trans*-fa-w-c complexes respectively confirm this hypothesis. The contributions to the non-zero values of  $P_c$  can be attributed to the out-of-plane water hydrogen atoms and to intermolecular vibration contributions. This is confirmed by the nearly unchanging value for  $P_c$  observed upon the isotopic substitution of both  $O_w$  and  $H_w$  atoms (see Tables S6 and S7). The *ab initio* calculations confirm this conclusion. The results of the  $r_s$  and  $r_0$  calculations for those complexes are collected in Tables S12-S13 and summarized in Figure 4. In those calculations the same considerations as for the *cis*-fa complexes were done.

The equilibrium conformation of the *trans*-fa-w-c complex seems to have  $C_s$  symmetry. *Trans*-formanilide subunit is planar, water oxygen atom lies in the symmetry plane and the water hydrogen atoms are expected to be out of the plane in symmetrical positions. The only stabilizing interaction is a  $O_w \cdots H_1-N$  hydrogen bond in which the molecule of water acts as hydrogen acceptor. The *ab initio* predictions locate the water oxygen atom in a position in which it can establish a weak interaction with the closest hydrogen atom from the phenyl ring. The agreement between calculated and experimental rotational parameters for this complex is worse than for the other analyzed complexes. This may be attributed to the fact that *ab initio* optimizations of the structure lead to a static picture of the complex which do not reflect the fact that water molecule in this complex is rotating with a low hindering barrier. The experimental values are expected values for the ground state, which may have important torsional contributions. This idea is reinforced by the  $P_c$  value for the <sup>18</sup>O<sub>w</sub> isotopologue (0.994 uÅ<sup>2</sup>, see Table S7), slightly smaller than the parent value, indicating that oxygen atom may be affected by some vibrational in-plane contribution

## Dynamics

In all the observed complexes the formamide subunit keeps the same planar (*trans*) or non-planar (*cis*) forms observed for the monomer.<sup>22</sup> Both detected *cis*-formamide complexes inherit the non-planar configuration of the formamide subunit observed in bare *cis*-formamide.

In the same context, the tunneling splittings observed for the *cis*-formamide monomer in the microwave spectrum reveal the existence of two non-planar equivalent conformers with a barrier of 152 cm<sup>-1</sup> at the planar *cis*-formamide form, estimated from a flexible one-dimensional model of the  $\angle C_1-N-C_2-C_3$  torsion.<sup>22</sup> For the *cis* 1:1 complex, closely spaced doublets in the limit of the MB-FTMW resolution were observed, while for the 1:2 complex no doublets were observed. These small doublets are also related to the existence of two equivalent non-planar conformers of *cis*-formamide...H<sub>2</sub>O. However, the mechanism of interconversion cannot be treated with the same one-dimensional model of the  $\angle C_1-N-C_2-C_3$  torsion as shown in Figure 5 where the one dimensional potential energy functions for this coordinate predicted for the monomer and the complex are compared.

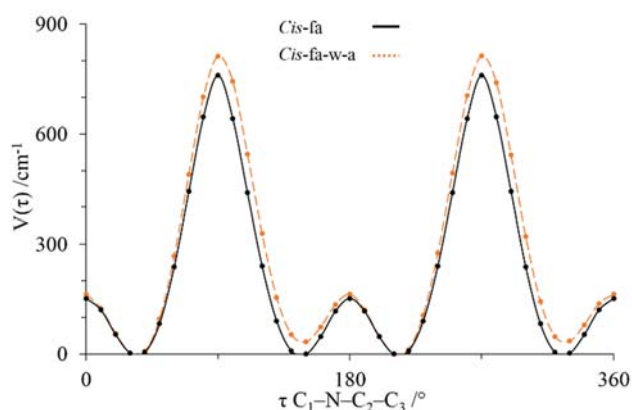


Figure 5. Periodic potential function for the  $C_1-N-C_2-C_3$  torsion of *cis*-fa monomer and *cis*-fa-w-a complex.

Theoretical calculations predict only small changes on the barriers to  $\angle C_1-N-C_2-C_3$  torsion at 0° and 90° but the minima around the planar *cis*-formamide forms are not equivalent. This gives rise to the *cis*-fa-w-a and *cis*-fa-w-a' forms, the former predicted to be more stable. Another motion, the flipping of water around the O-H...O hydrogen bond axis interconverts *cis*-fa-w-a' and *cis*-fa-w-a' forms (see Figure S5). In fact, the interconversion between the equivalent *cis*-fa-w-a forms would involve a concerted motion of the  $\angle C_1-N-C_2-C_3$  torsion and the flipping motion of water, which would increase additionally the torsional barriers. The decrease in the torsional splitting observed for *cis*-formamide...H<sub>2</sub>O with respect to *cis*-formamide can then be attributed to that barrier increment together with the increased reduced mass due to the presence of water in the rotating group. The non-observation of the low energy conformer *cis*-fa-w-a' in the supersonic expansion can be attributed to collision conformational relaxation in the supersonic jet to the global minimum conformation.<sup>42</sup> Any of the two possible paths;

water flipping or  $\angle C_1-N-C_2-C_3$  torsion, have barriers low enough to allow for such a relaxation. Similar conclusions can be drawn for the dihydrated *cis*-formamide complex.

The observation of doublets in the spectrum of *trans*-fa-w-c form should be also discussed. The fact that the observed doublets show statistical weight spin effects due to the exchange of two hydrogen atoms reveals that the associated tunneling motion is related to the internal rotation of the H<sub>2</sub>O molecule around its local C<sub>2</sub> axis. *Ab initio* calculations along the water rotation angle reveal a two-fold periodical potential energy function with barriers of 312 cm<sup>-1</sup> (B3LYP-D3/6-311++G(d,p)) or 349 cm<sup>-1</sup> (MP2/6-311++G(2d,p)) at the planar configuration where H...H repulsive interactions may take place (see Figure 6). Flexible model calculations<sup>43</sup> using a simple rotation of water around its C<sub>2</sub> axis show that the value  $\Delta P_{cc} = 0.095 \text{ u\AA}^2$ , measuring the change in the  $P_{cc}$  planar moment in going for the  $v=0$  to the  $v=1$  internal rotation states, is reproduced for  $V_2=193 \text{ cm}^{-1}$ . The same dynamics was observed for the related complexes formamide-H<sub>2</sub>O I<sub>c</sub> ( $V_2=117 \text{ cm}^{-1}$ )<sup>16</sup> and in indole...H<sub>2</sub>O ( $V_2=182 \text{ cm}^{-1}$ ).<sup>31</sup>

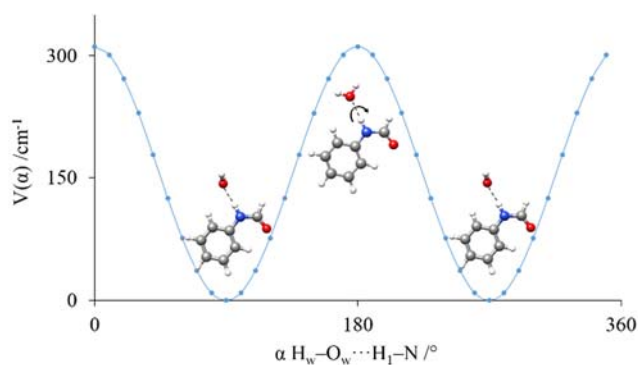


Figure 6. Periodic potential function for the internal rotation of water molecule in the *trans*-fa-w-c complex.

## Discussion

The  $r_0$  structures observed are comparable to those of similar microsolvated complexes modeling the peptide group like formamide,<sup>16</sup> *trans*-N-methyl formamide,<sup>5</sup> azetidinone<sup>41</sup> or 2-pyridone.<sup>19</sup> As shown in Tables S10-S13 they compare very well with the microsolvated complexes of formamide, which as formamide cover all possible interactions observable between water and the *cis* or *trans* forms of peptide group. In all cases, the formamide group and the interacting water atoms are nearly coplanar. The hydrogen bond distances, which can be related to the strength of the interactions, reveal interesting details. In the 1:1 complexes the C=O...H-O bond distances are of the same order of magnitude and the same can be said of the N-H...O distance. The shorter distance corresponds to the C=O...H-O interaction which could be an indication of its dominant character in good agreement with the preference of water to interact with the carbonyl group observed in globular proteins.<sup>3</sup>

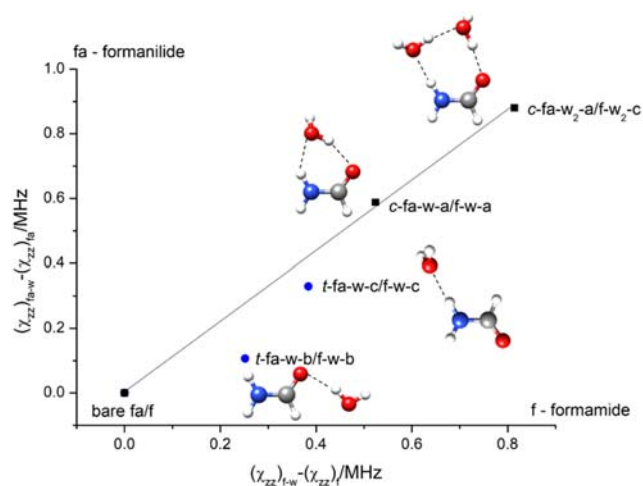


Figure 7. Correlation between the values of the quadrupole coupling constant  $\chi_{zz}$  of formamide-water and formanilide-water complexes. The values of  $\chi_{zz}$  relative to the monomer values are represented. The values corresponding to the *cis*-formanilide-water 1:1 and 1:2 complexes lie in a straight line showing the effects of  $\pi$ -cooperativity.<sup>17,18</sup>

The signatures of sigma bond cooperativity<sup>2,44</sup> can be seen in the shortening of the hydrogen bond distances observed in the 1:2 with respect to the 1:1 complexes. These distances are observed to decrease as the number of water molecules increases,<sup>16,18</sup> as an important characteristic of sequential cycles.<sup>2</sup> A second signature of this kind of cooperativity can be obtained by analyzing the dissociation energies per hydrogen bond of the *cis*-formanilide complexes. The comparison of the total dissociation energies per bond, 33.2 kJ/mol for *cis*-fa-w<sub>2</sub>-a and 23.2 kJ/mol for *cis*-fa-w-a, indicates the reinforcement of the hydrogen bond interactions when increasing the number of cooperative bonds. The dissociation energies (see Tables S1-S4) indicate, on the other hand, that the *cis*-formanilide complexes formed by sequential cycles reinforced by cooperativity are more stable than the *trans*-formanilide complexes.

Resonance assisted hydrogen bonding (RAHB) or  $\pi$ -cooperativity polarizes the amide group  $\pi$ -electron distribution and causes the C=O bond enlargement and C-N bond shortening. These structural changes, first pointed out to occur in chains of small amides<sup>44,45</sup> have been detected only in few cases in gas phase<sup>17-19</sup> and are predicted to occur also for the formanilide complexes (see Table S14). It has been recently shown for the series of formamide-water complexes<sup>17,18</sup> that the <sup>14</sup>N quadrupole coupling constants can be taken as a probe for the polarization effects associated to these structural changes since it gives information about the electronic environment around the N atom. In particular, the planarity of formamide-water complexes has allowed to show that the steady variation of the  $\chi_{cc}$  constant when increasing the number of interacting water molecules can be associated with the expected progressive depletion of the electron population of the N atom lone pair. This behavior can thus be taken as a signature of  $\pi$ -cooperativity. The non-planarity of *cis*-formanilide does not allow us to take directly the same approximations as in formamide. However, by considering the

good agreement between the experimental and predicted quadrupole coupling constants we have tested the behavior of the predicted principal quadrupole coupling constant. As can be seen in Figure 7 the  $\chi_{zz}$  constants for the *cis*-formanilide water complexes follow the same behavior as observed in formamide water complexes.<sup>16-18</sup> Figure S6 shows also the correlation between the predicted  $\chi_{zz}$  constant values and those for the  $r_e(\text{C-N})$  and  $r_e(\text{C=O})$  distances. This allows us to conclude that resonance assisted hydrogen bond inductive effects also play in the formanilide microsolvates to almost the same extent as happens in formamide-water complexes. One may wonder also if these RAHB effects have some influence in the potential energy function of the  $\angle\text{C}_1\text{-N-C}_2\text{-C}_3$  torsion of *cis*-formanilide and on the corresponding equilibrium angles, which show also a steady small decrease with increasing hydration number.

## 4 Conclusions

To conclude, this work illustrates the preferred interactions sites of water with formanilide and the stabilization interactions established for the different complexes formed. This work shows that *cis*-formanilide-water complexes are also present in supersonic jets with enough abundance to show intense rotational spectra, contrary to the conclusions of previous works.<sup>23,24,26,27,29</sup> Thus, it complements those previous studies and the microsolvation of the amide moiety in general,<sup>15-19</sup> providing another model to understand the solvation of the peptide bond. The way in which water interacts with the amide group is very different for the *cis* and *trans* forms of formanilide. In the *cis*-formanilide configuration the amino group (N-H) and carbonyl group (C=O) are on the same side. These two groups can interact simultaneously with one or several water molecules forming sequential cycles<sup>16</sup> as those observed for *cis*-formanilide $\cdots\text{H}_2\text{O}$  and *cis*-formanilide $\cdots(\text{H}_2\text{O})_2$ . However for the *trans* form, which models the *trans*-peptide arrangement, the amino and carbonyl groups are pointing in opposite sides of the amide group so water cannot interact with both simultaneously as occur for the *cis* form. In this way, we have observed two different complexes. The *trans*-formanilide $\cdots\text{H}_2\text{O}$ -b complex is formed through an O-H $\cdots$ O=C hydrogen bond with water, and is further stabilized by a weak C-H $\cdots$ O contact. The complex *trans*-formanilide $\cdots\text{H}_2\text{O}$ -c, is formed by a N-H $\cdots$ O hydrogen bond but water do not establishes any other contact and thus is able to rotate around the hydrogen bond axis. A small two-fold barrier to this motion splits the rotational transitions in two tunneling states and exchanges the water hydrogen atoms so that the corresponding statistical spin weight effects are reflected in the intensities of the observed splittings.

The structures of these two *trans*-formanilide $\cdots\text{H}_2\text{O}$  complexes can be useful to describe the way in which water may interact with the *trans* peptide groups, overwhelmingly favored in proteins and peptides. Water may establish either N-H $\cdots$ O or O-H $\cdots$ O=C bonds but cannot completely satisfy its multiple donor/acceptor capacity since it is in a hydrophobic environment. These interactions may help in the formation of

N-H...O=C interactions,<sup>17</sup> which dominate in the core of proteins.<sup>46</sup> The *cis* form of the peptide group is found principally in residues involving proline so the *cis*-formamide-H<sub>2</sub>O interactions observed here would be rarely observed in proteins peptide chains. However, these could be observed for residues as those of asparagine and glutamine having a formamide group in the side chains. These two residues are often found at the C-terminus in  $\alpha$ -helices.<sup>47</sup> It has been pointed out that these primary amide...(H<sub>2</sub>O)<sub>n</sub> cyclic structures have a key role in the deamidation of these residues.<sup>48</sup> Another conclusion that can be extracted from this work is the alteration of the *cis/trans* conformational equilibrium of formamide by its interaction with water. *Ab initio* calculations predict the inversion of the relative stability of the two forms by complexation with water. For bare formamide, the *trans* conformer is more stable. In the 1:1 complexes, the most stable conformer corresponds to the *cis* form, being the complexes with the *trans* species higher in energy. The difference in energy increases when considering dihydrated complexes (see Tables S1-S4). This is in agreement with the species observed experimentally in this work. For 1:1 complexes, both the most stable *cis*-fa-w-a and the higher energy *trans*-fa-w-b and *trans*-fa-w-c forms are present. However, for the 1:2 complexes, the *cis*-form predominates and no dihydrated complexes with *trans*-formamide have been observed.

## Conflicts of interest

Authors declare that there are no conflicts of interest.

## Acknowledgments

The authors acknowledge the Ministerio de Economía y Competitividad (Grant CTQ2016-75253-P) and the Junta de Castilla y León (Grant VA334U14) for financial support.

## References

- W. Saenger, G. A. Jeffrey, *Hydrogen Bonding in Biological Structures*, Springer-Verlag, Berlin 1991.
- G. A. Jeffrey, *An Introduction to Hydrogen Bonding*, Oxford University Press, Oxford, 1997.
- E. N. Baker, R. E. Hubbard, *Prog. Biophys. Mol. Biol.*, 1984, **44**, 97–179.
- M. Schmitt, M. Böhm, C. Ratzer, C. Vu, I. Kalkman, W. L. Meerts, *J. Am. Chem. Soc.*, 2005, **127**, 10356–10364.
- W. Caminati, J. C. López, S. Blanco, S. Mata, J. L. Alonso, *Phys. Chem. Chem. Phys.*, 2010, **12**, 10230–10234.
- A. Maris, P. Ottaviani, W. Caminati, *Chem. Phys. Lett.*, 2002, **360**, 155–160.
- S. Mata, V. Cortijo, W. Caminati, J. L. Alonso, M. E. Sanz, J. L. López, S. Blanco, *J. Phys. Chem. A*, 2010, **114**, 11393–11398.
- B. L. Groot, H. Grubmüller, *Science*, 2001, **294**, 2353–2357.
- P. Ball, *Chem. Rev.*, 2008, **108**, 74–108.
- O. Dopfer, M. Fujii, *Chem. Rev.*, 2016, **116**, 5432–5463.
- M. Becucci, S. Melandri, *Chem. Rev.*, 2016, **116**, 5014–5037.
- C. Pérez, M. T. Muckle, D. P. Zaleski, N. A. Seifert, B. Temelso, G. C. Shields, Z. Kisiel, B. H. Pate, *Science*, 2012, **336**, 897–901.
- C. Pérez, J. C. López, S. Blanco, M. Schnell, *J. Phys. Chem. Lett.*, 2016, **7**, 4053–4058.
- C. Pérez, A. Krin, A. L. Steber, J. C. López, Z. Kisiel, M. Schnell, *J. Phys. Chem. Lett.*, 2016, **7**, 154–160.
- F. J. Lovas, R. D. Suenram, G. T. Fraser, C. W. Gillies, J. Zozom, *J. Chem. Phys.*, 1988, **88**, 722–729.
- S. Blanco, J. C. López, A. Lesarri, J. L. Alonso, *J. Am. Chem. Soc.*, 2006, **128**, 12111–12121.
- S. Blanco, P. Pinacho, J. C. López, *Angew. Chem. Int. Ed.*, 2016, **128**, 9477–9481.
- S. Blanco, P. Pinacho, J. C. López, *J. Phys. Chem. Lett.*, 2017, **8**, 6060–6066.
- A. Held, D. W. Pratt, *J. Am. Chem. Soc.*, 1993, **115**, 9708–9717.
- V. P. Manea, K. J. Wilson, J. R. Cable, *J. Am. Chem. Soc.*, 1997, **119**, 2033–2039.
- P. Ottaviani, S. Melandri, A. Maris, P. G. Favero, W. Caminati, *J. Mol. Spectrosc.*, 2001, **205**, 173–176.
- S. Blanco, J. C. López, A. Lesarri, W. Caminati, J. L. Alonso, *Mol. Phys.*, 2005, **103**, 1473–1479.
- J. A. Dickinson, M. R. Hockridge, E. G. Robertson, J. P. Simons, *J. Phys. Chem. A*, 1999, **103**, 6938–6949.
- A. V. Fedorov, J. R. Cable, *J. Phys. Chem. A*, 2000, **104**, 4943–4952.
- E. G. Robertson, *Chem. Phys. Lett.*, 2000, **325**, 299–307.
- M. Mons, I. Dimicoli, B. Tardivel, F. Piuze, E. G. Robertson, J. P. Simons, *J. Phys. Chem. A*, 2001, **105**, 969–973.
- S. Ullrich, G. Tarczay, X. Tong, C. E. H. Dessent, K. Müller-Dethlefs, *Phys. Chem. Chem. Phys.*, 2001, **3**, 5450–5458.
- J. R. Clarkson, E. Baquero, V. A. Shubert, E. M. Myshakin, K. D. Jordan, T. S. Zwier, *Science*, 2005, **307**, 1443–1446.
- K. Sakota, Y. Shimazaki, H. Sekiya, *Phys. Chem. Chem. Phys.*, 2011, **13**, 6411–6415.
- a) G. G. Brown, B. C. Dian, K. O. Douglass, S. M. Geyer, B. H. Pate, *J. Mol. Spectrosc.*, 2006, **238**, 200–212. b) G. G. Brown, B. C. Dian, K. O. Douglass, S. M. Geyer, S. T. Shipman, B. H. Pate, *Rev. Sci. Instrum.*, 2008, **79**, 053103.
- S. Blanco, J. C. López, J. L. Alonso, P. Ottaviani, W. Caminati, *J. Chem. Phys.*, 2003, **119**, 880–886.
- J. L. Alonso, F. J. Lorenzo, J. C. López, A. Lesarri, S. Mata, H. Dreizler, *Chem. Phys.*, 1997, **218**, 267–275.
- Gaussian 09, Revision D.01, Frisch M. J., Trucks G. W., Schlegel H. B., Scuseria G. E., Robb M. A., Cheeseman J. R., Scalmani G., Barone V., Petersson G. A., Nakatsuji H., Li X., Caricato M., Marenich A., Bloino J., Janesko B. G., Gomperts R., Mennucci B., Hratchian H. P., Ortiz J. V., Izmaylov A. F., Sonnenberg J. L., Williams-Young D., Ding F., Lipparini F., Egidi F., Goings J., Peng B., Petrone A., Henderson T., Ranasinghe D., Zakrzewski V. G., Gao J., Rega N., Zheng G., Liang W., Hada M., Ehara M., Toyota K., Fukuda R., Hasegawa J., Ishida M., Nakajima T., Honda Y., Kitao O., Nakai H., Vreven T., Throssell K., Montgomery, Jr. J. A., Peralta J. E., Ogliaro F., Bearpark M., Heyd J. J., Brothers E., Kudin K. N., Staroverov V. N., Keith T., Kobayashi R., Normand J., Raghavachari K., Rendell A., Burant J. C., Iyengar S. S., Tomasi J., Cossi M., Millam J. M., Klene M., Adamo C., Cammi R., Ochterski J. W., Martin R. L., Morokuma K., Farkas O., Foresman J. B., Fox D. J., Gaussian, Inc., Wallingford CT, 2016.
- H. M. Pickett, *J. Mol. Spectrosc.*, 1991, **148**, 371–377.
- J. K. G. Watson, in *Vibrational Spectra and Structure a Series of Advances*, Vol 6, ed. J. R. Durig, Elsevier, New York, 1977, pp. 1–89.
- W. Gordy, R. L. Cook, *Microwave molecular spectra*, Wiley-Interscience, New York, 1972, vol. 11.
- J. Kraitchman, *Am. J. Phys.*, 1953, **21**, 17–24.
- H. D. Rudolph, *Struct. Chem.*, 1991, **2**, 581–588.
- Z. Kisiel, *J. Mol. Spectrosc.*, 2003, **218**, 58–67.



- <sup>40</sup> C. C. Costain, *Trans. Am. Crystallogr. Assoc.*, 1966, **2**, 157–164.
- <sup>41</sup> J. C. López, R. Sanchez, S. Blanco, J. L. Alonso, *Phys. Chem. Chem. Phys.*, 2015, **17**, 2054–2066.
- <sup>42</sup> R. S. Ruoff, T.D. Klots, T. Emilson, H.S. Gutowski, *J. Chem. Phys.*, 1990, **93**, 3142–3150.
- <sup>43</sup> R. Meyer, *J. Mol. Spectrosc.*, 1979, **76**, 266–300.
- <sup>44</sup> P. Gilli, V. Bertolasi, V. Ferretti, G. Gilli, G., *J. Am. Chem. Soc.*, 2000, **122**, 10405–10417.
- <sup>45</sup> T. Ottersen, *Acta Chemica Scandinavica*, 1975, **29a**, 939–944.
- <sup>46</sup> C. M. Venkatachalam, *Biopolymers*, 1968, **6**, 1425–1436.
- <sup>47</sup> J. S. Richardson, D. C. Richardson, *Science*, 1988, **240**, 1648–1652.
- <sup>48</sup> S. Catak, G. Monard, V. Aviyente, M. F. Ruiz-López, *J. Phys. Chem. A*, 2009, **113**, 1111–1120.