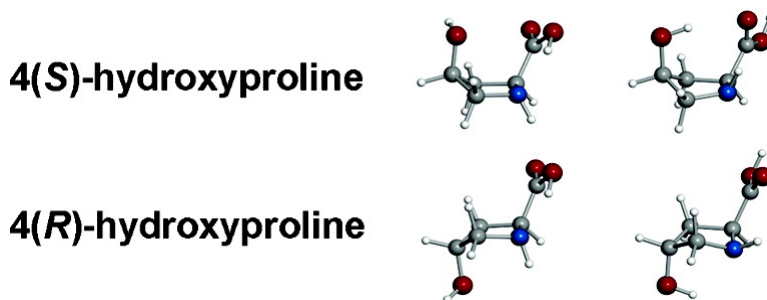


## Shape of 4(*S*)- and 4(*R*)-Hydroxyproline in Gas Phase

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## Shape of 4(S)- and 4(R)-Hydroxyproline in Gas Phase

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**Abstract:** The  $\alpha$ -amino acids 4(S)-hydroxyproline and 4(R)-hydroxyproline have been studied under isolation conditions in gas phase using laser-ablation molecular-beam Fourier transform microwave spectroscopy. Two conformers of each molecule have been detected in the jet-cooled rotational spectrum. The most stable conformer in both molecules exhibits an intramolecular N $\cdots$ H–O hydrogen bond (configuration 1) between the hydrogen atom of the carboxylic group and the nitrogen atom. The second conformer is characterized by an intramolecular N–H $\cdots$ O=C hydrogen bond (configuration 2). The conformers of 4(R)-hydroxyproline adopt a C $\gamma$ -*exo* puckering, while those of 4(S)-hydroxyproline present a C $\gamma$ -*endo* ring conformation. These ring conformations, which show the same propensity observed in collagen-like peptides, are stabilized by additional intramolecular hydrogen bonds involving the 4-hydroxyl group, with the exception of the most stable form of 4(S)-hydroxyproline for which a n– $\pi^*$  interaction between the oxygen atom of the 4-hydroxyl group and the carboxyl group carbon seems to be established. A gauche effect could be also contributing to stabilize the observed conformers.

## Introduction

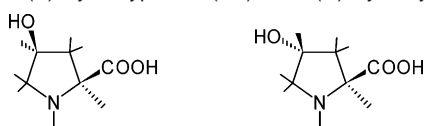
Spectroscopic studies of  $\alpha$ -amino acids (NH $_2$ –CH(R)–COOH) in gas phase are directed to the conformational and structural analysis of the neutral forms of these building blocks of life, providing a picture of their intrinsic molecular properties free of intermolecular interactions.<sup>1</sup> Amino acids are particularly appealing because of the great number of low-energy conformers, the result of a great torsional flexibility combined with very delicate energy balances among different noncovalent interactions, especially intramolecular hydrogen bonding. Though some conformational studies of natural amino acids with aromatic chromophores (phenylalanine,<sup>2</sup> tyrosine,<sup>2a,3</sup> tryptophan<sup>4</sup>) have been carried out using electronic spectroscopy, detailed structural information is only available from their rotational spectra. However, the structural analysis of natural amino acids using rotational spectroscopy was hindered in the past by the high melting points and thermal fragility of these compounds, and only glycine<sup>5</sup> and alanine<sup>6</sup> were observed and characterized in

gas phase, together with some amide derivatives.<sup>7</sup> Recently, these studies have received an important impulse with the introduction by our group of a technique which combines laser-ablation with Fourier transform microwave spectroscopy in supersonic jets<sup>8</sup> (laser-ablation molecular-beam FT-microwave spectroscopy, LA-MB-FTMW). Using this technique, we have studied proline,<sup>9</sup> valine<sup>10</sup> and alanine,<sup>11</sup> and other organic solids, such as thiourea.<sup>12</sup>

Rotational spectroscopy in supersonic jets has confirmed that the gas phase conformational preferences of small  $\alpha$ -amino acids (NH $_2$ –CH(R)–COOH) with a nonpolar aliphatic side chain (R) depend primarily on the formation of intramolecular hydrogen bonds between the polar groups of the  $\alpha$ -amino acid skeleton. In the cases of glycine<sup>5</sup> (R = H), alanine<sup>6,11</sup> (R = CH $_3$ ), and valine<sup>10</sup> (R = CH(CH $_3$ ) $_2$ ), the bifurcated amine-to-carbonyl

- (1) Robertson, E. G.; Simons, J. P. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1. (b) Weinkauff, R.; Schermann, J.-P.; de Vries, M. S.; Kleinermans, K. *Eur. Phys. J. D* **2002**, *20*, 309. (c) Zwier, T. S. *J. Phys. Chem. A* **2001**, *105*, 8827.
- (2) Martinez, S. J., III; Alfano, J. C.; Levy, D. H. *J. Mol. Spectrosc.* **1992**, *156*, 421. (b) Snoek, L. C.; Robertson, E. G.; Kroemer, R. T.; Simons, J. P. *Chem. Phys. Lett.* **2000**, *321*, 49.
- (3) Cohen, R.; Brauer, B.; Nir, E.; Grace, L.; de Vries, M. S. *J. Phys. Chem. A* **2000**, *104*, 6351. (b) Grace, L. I.; Cohen, R.; Dunn, T. M.; Lubman, D. M.; de Vries, M. S. *J. Mol. Spectrosc.* **2002**, *215*, 204.
- (4) Rizzo, T. R.; Park, Y. D.; Peteanu, L. A.; Levy, D. H. *J. Chem. Phys.* **1985**, *83*, 4819. (b) Rizzo, T. R.; Park, Y. D.; Peteanu, L. A.; Levy, D. H. *J. Chem. Phys.* **1986**, *84*, 2534. (c) Rizzo, T. R.; Park, Y. D.; Levy, D. H. *J. Chem. Phys.* **1986**, *85*, 6945. (d) Piuze, F.; Dimicoli, L.; Mons, M.; Tardivel, B.; Zhao, Q. *Chem. Phys. Lett.* **2000**, *320*, 282. (e) Snoek, L. C.; Kroemer, R. T.; Hockridge, M. R.; Simons, J. P. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1819. (f) Bakker, J. M.; Aleese, L. M.; Meijer, G.; von Helden, G. *Phys. Rev. Lett.* **2003**, *91*, 203003.

- (5) Brown, R. D.; Godfrey, P. D.; Storey, J. W. V.; Bassez, M. P. *J. Chem. Soc., Chem. Commun.* **1978**, 547. (b) Suenram, R. D.; Lovas, F. J. *J. Mol. Spectrosc.* **1978**, *72*, 372. (c) Suenram, R. D.; Lovas, F. J. *J. Am. Chem. Soc.* **1980**, *102*, 7180. (d) Lovas, F. J.; Kawashima, Y.; Grabow, J.-U.; Suenram, R. D.; Fraser, G. T.; Hirota, E. *Astrophys. J.* **1995**, *455*, L201. (e) Godfrey, P. D.; Brown, R. D. *J. Am. Chem. Soc.* **1995**, *117*, 2019. (f) McGlone, S. J.; Elmes, P. S.; Brown, R. D.; Godfrey, P. D. *J. Mol. Struct.* **1999**, *485*, 225.
- (6) Godfrey, P. D.; Firth, S.; Hatherley, L. D.; Brown, R. D.; Pierlot, A. P. *J. Am. Chem. Soc.* **1993**, *115*, 9687.
- (7) Lavrich, R. J.; Plusquellic, D. F.; Suenram, R. D.; Fraser, G. T.; Hight Walker, A. R.; Tubergen, M. J. *J. Chem. Phys.* **2003**, *118*, 1253. (b) Kuhls, K. A.; Centrone, C. A.; Tubergen, M. J. *J. Am. Chem. Soc.* **1998**, *120*, 10194.
- (8) Lesarri, A.; Mata, S.; López, J. C.; Alonso, J. L. *Rev. Sci. Instrum.* **2003**, *74*, 4799.
- (9) Lesarri, A.; Mata, S.; Cocinero, E. J.; Blanco, S.; López, J. C.; Alonso, J. L. *Angew. Chem., Int. Ed.* **2002**, *41*, 4673.
- (10) Lesarri, A.; Cocinero, E. J.; López, J. C.; Alonso, J. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 605.
- (11) Blanco, S.; Lesarri, A.; López, J. C.; Alonso, J. L. *J. Am. Chem. Soc.* **2004**, *126*, 11675.
- (12) Lesarri, A.; Mata, S.; Blanco, S.; López, J. C.; Alonso, J. L. *J. Chem. Phys.* **2004**, *120*, 6191.

**Scheme 1.** 4(*S*)-Hydroxyproline (left) and 4(*R*)-Hydroxyproline

interaction ( $N-H\cdots O=C$ , configuration 2) gives rise to the most stable conformers, with independence of the size of the different side chains. In these three cases, it has been observed that the second stable conformer is stabilized by an intramolecular hydrogen bond between the hydrogen atom of the carboxyl group and the nitrogen atom lone pair ( $N\cdots H-O$ , configuration 1). A plausible configuration 3 stabilized by a  $N-H\cdots O-H$  hydrogen bond has not been observed in the jet-cooled rotational spectra of glycine,<sup>5</sup> alanine,<sup>6,11</sup> or valine.<sup>10</sup> Proline is a singular example among natural amino acids because its side chain is closing a pyrrolidine ring, resulting in a unique imino functionality. Two conformers of neutral proline were observed in the supersonic jet,<sup>9</sup> which exhibit an intramolecular  $N\cdots H-O$  hydrogen bond (configuration 1). The flexible pyrrolidine ring in proline can adopt two bent conformations, either  $C_\gamma$ -endo (conformer 1a, global minimum) or  $C_\gamma$ -exo (conformer 1b). Conformers with an intramolecular  $N-H\cdots O=C$  hydrogen bond (configuration 2), an analogue to those observed for the most stable conformers of glycine, alanine, and valine, were not detected for proline.

In this work, we present the first study of isolated neutral 4(*S*)- and 4(*R*)-hydroxyproline in gas phase (Scheme 1). There is no previous structural information on these molecules apart from a crystal structure study on the zwitterionic form of 4(*R*)-hydroxyproline.<sup>13</sup> Proline and 4(*R*)-hydroxyproline are of biological relevance since they are the major constituents of collagen, the most abundant protein in vertebrates. Collagen<sup>14</sup> is a triple helix made of three super-coiled polyproline II-like chains<sup>15</sup> with repetitive tripeptide sequences,  $X-Y-Gly$ , where usually  $X = \text{proline}$  and  $Y = 4(R)\text{-hydroxyproline}$ . The triple helix structure produces a great tensile strength appropriate for force transmission, as in tendons, or for shielding structures, as in the skin. 4(*R*)-Hydroxyproline is originated from enzymatic hydroxylation of proline residues after collagen biosynthesis, before forming the triple helix.<sup>14,15</sup> This post-translational event is both position-dependent and stereoselective. In vertebrate collagen, only proline molecules in the *Y* position are hydroxylated, and only the 4(*R*)-hydroxyproline diastereoisomer results from this process. Hydroxylation in the *Y* position contributes significantly to the thermal stability of the triple helix.<sup>16</sup> On the other hand, the diastereoisomer 4(*S*)-hydroxyproline inhibits<sup>17</sup> the proper folding of the triple helix either in the *X* or *Y*

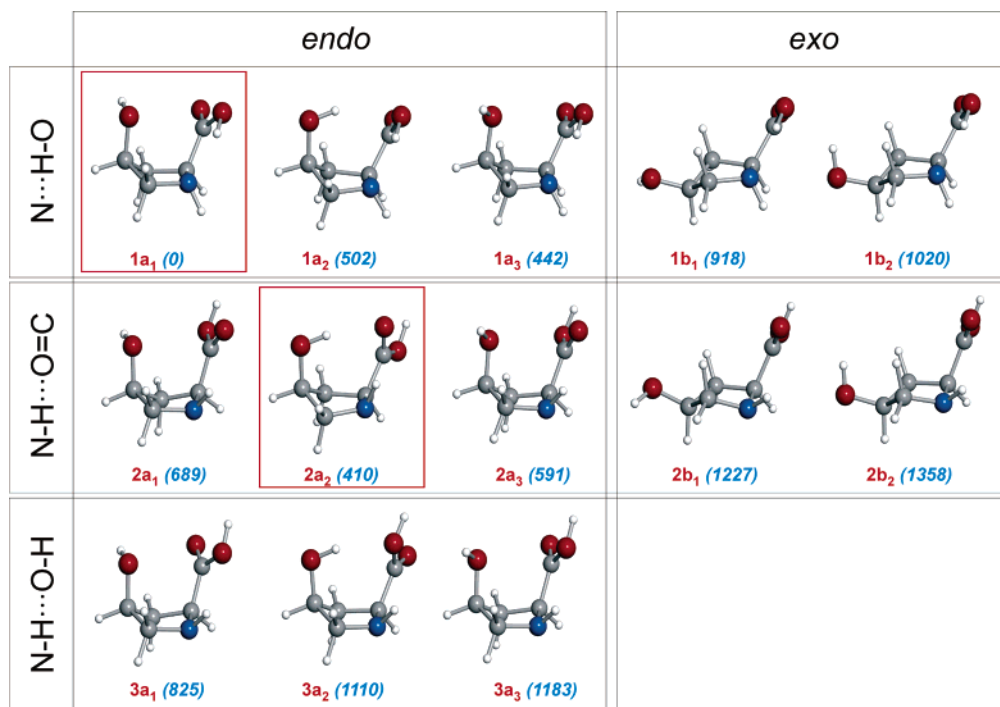
position. Several contrasting models (water-mediated hydrogen bonds,<sup>18</sup> inductive effects,<sup>19</sup> or stereochemical interactions<sup>20</sup>) explain the opposing stabilization effects caused by 4(*S*)- and 4(*R*)-hydroxyproline, which are still not fully understood.<sup>21</sup> In particular, Raines<sup>19</sup> and co-workers suggest that collagen stability could be related to the conformational preferences of 4(*R*)-hydroxyproline through a stereoelectronic<sup>22</sup> effect originated by the introduction of an electronegative  $-OH$  substituent in the  $C_\gamma$  position of the ring. According to this view, the electron-withdrawing capability of the 4-hydroxy group would cause a so-called "gauche effect"<sup>19,23</sup> in the  $O-C_\gamma-C_\delta-N$  chain, resulting in a preference for the maximum number of gauche interactions between the adjacent  $O-C_\gamma$  and  $C_\delta-N$  polar bonds. The 4-hydroxy group can also produce additional hydrogen bonding within these molecules, affecting their conformational landscapes.

A comparative study of the intrinsic properties of 4(*S*)- and 4(*R*)-hydroxyproline in the isolated environment of a supersonic jet will serve to examine what conformational changes are induced in proline by the introduction of a 4-hydroxy group. Experimental information on the extent and magnitude of the different interactions which govern the structure of these pyrrolidine-containing amino acids could help to understand their dynamical role in collagen biosynthesis.

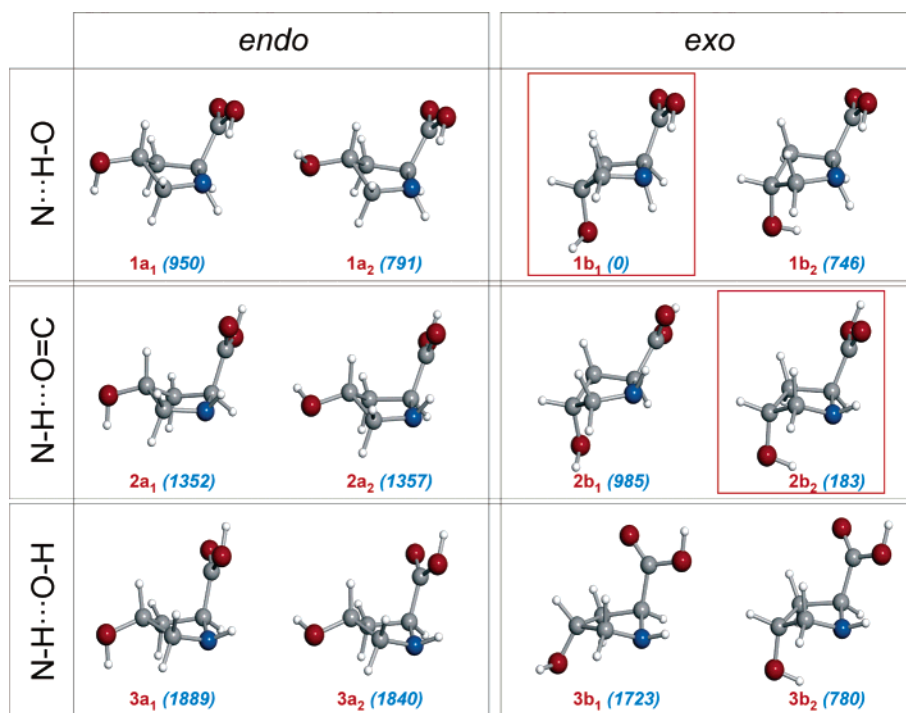
## Experimental and Theoretical Methods

**Laser-Ablation and Rotational Spectroscopy.** The laser-ablation molecular-beam Fourier transform microwave spectrometer (LA-MB-FTMW) has been described elsewhere.<sup>8</sup> This instrument has been modified recently with the construction of a larger high-vacuum chamber to accommodate enlarged Fabry-Pérot mirrors (55 cm diameter) and an auxiliary chamber to facilitate removing of samples without stopping the vacuum system. 4(*S*)-Hydroxyproline and 4(*R*)-hydroxyproline (mp 243 and 273°, respectively) were obtained commercially (99%) and used without further purification. The mono-deuterated derivatives ( $-COOD$ ,  $-ND$ , and  $-OD$ ) were prepared dissolving either 4(*S*)- or 4(*R*)-hydroxyproline in  $D_2O$ , followed by recrystallization. The solid samples of 4(*S*)- or 4(*R*)-hydroxyproline were finely pulverized and pressed to form 6 mm diameter bars 1–2 cm long. Minimum quantities (1–3 drops/g) of a binder were used to improve the mechanical stability of the sample. The sample targets are held vertically on a special nozzle located on the backside of one of the mirrors forming the Fabry-Pérot resonator of the spectrometer. For each of the molecular pulses (typically 0.5–0.7 ms), the solid

- (13) Donohue, J.; Trueblood, K. N. *Acta Crystallogr.* **1952**, *5*, 414. (b) Donohue, J.; Trueblood, K. N. *Acta Crystallogr.* **1952**, *5*, 419. (c) Koetzle, T.; Lehmann, M. S.; Hamilton, W. C. *Acta Crystallogr.* **1973**, *B29*, 231.
- (14) *Biochemistry of Collagen*; Ramachandran, G. N., Reddi, A. H., Eds.; Plenum Press: New York, 1976. (b) *Collagen*; Nimmi, M. E., Ed.; CRC Press: Boca Raton, FL, 1988; Vols. 1–4. (c) Adams, E.; Frank, L. *Annu. Rev. Biochem.* **1980**, *49*, 1005.
- (15) Ramachandran, G. N.; Kartha, G. *Nature* **1955**, *176*, 593. (b) Rich, A.; Crick, F. H. C. *J. Mol. Biol.* **1961**, *3*, 483. (c) Fraser, R. D.; McRae, T. P.; Suzuki, E. *J. Mol. Biol.* **1979**, *129*, 463. (d) Beck, K.; Brodsky, B. *J. Struct. Biol.* **1998**, *122*, 17.
- (16) Berg, R. A.; Prockop, D. J. *Biochem. Biophys. Res. Commun.* **1973**, *52*, 115. (b) Chopra, R. K.; Ananthanarayanan, V. S. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 7180. (c) Privalov, P. L. *Adv. Protein Chem.* **1982**, *35*, 1. (d) Sakakibara, S.; Inouye, K.; Shudo, K.; Kishida, Y.; Kobayashi, Y.; Prockop, D. J. *Biochim. Biophys. Acta* **1973**, *303*, 198. (e) Inouye, K.; Kobayashi, Y.; Kyogoku, Y.; Kishida, Y.; Sakakibara, S.; Prockop, D. J. *Arch. Biochem. Biophys.* **1982**, *219*, 198.
- (17) Rosenbloom, J.; Prockop, D. J. *J. Biol. Chem.* **1971**, *246*, 1549. (b) Inouye, K.; Sakakibara, S.; Prockop, D. J. *Biochim. Biophys. Acta* **1976**, *420*, 133.
- (18) Bella, J.; Eaton, M.; Brodsky, H. M. *Science* **1994**, *266*, 75. (b) Bella, J.; Brodsky, B.; Berman, H. *Structure* **1995**, *3*, 893. (c) Cramer, R. Z.; Berman, H. M. *J. Biomol. Struct. Dyn.* **1998**, *16*, 367.
- (19) Holmgren, S. K.; Taylor, K. M.; Bretscher, L. E.; Raines, R. T. *Nature* **1998**, *392*, 666. (b) Holmgren, S. K.; Bretscher, L. E.; Taylor, K. M.; Raines, R. T. *Chem. Biol.* **1999**, *6*, 63. (c) Bretscher, L. E.; Jenkins, C. L.; Taylor, K. M.; DeRider, M. L.; Raines, R. T. *J. Am. Chem. Soc.* **2001**, *123*, 777. (d) DeRider, M. L.; Wilkens, S. J.; Waddell, M. J.; Bretscher, L. E.; Weinhold, F.; Raines, R. T.; Markley, J. L. *J. Am. Chem. Soc.* **2002**, *124*, 2497. (e) Hodges, J. A.; Raines, R. T. *J. Am. Chem. Soc.* **2003**, *125*, 9262.
- (20) Vitagliano, L.; Berisio, R.; Mazzarella, L.; Zagari, A. *Biopolymers* **2001**, *58*, 459. (b) Berisio, R.; Vitagliano, L.; Mazzarella, L.; Zagari, A. *Protein Sci.* **2002**, *11*, 262. (c) Improtta, R.; Benzi, C.; Barone, V. *J. Am. Chem. Soc.* **2001**, *123*, 12568. (d) Improtta, R.; Mele, F.; Crescenzi, O.; Benzi, C.; Barone, V. *J. Am. Chem. Soc.* **2002**, *124*, 7857.
- (21) Jenkins, C. L.; Raines, R. T. *Nat. Prod. Rep.* **2002**, *19*, 49.
- (22) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon Press: New York, 1983. (b) Thatcher, G. R. J. *The Anomeric Effect and Associated Stereoelectronic Effects*; American Chemical Society: Washington, DC, 1993.
- (23) Benzi, C.; Improtta, R.; Scalmani, G.; Barone, V. *J. Comput. Chem.* **2002**, *23*, 341. (b) Mooney, S. D.; Kollman, P. A.; Klein, T. E. *Biopolymers* **2002**, *64*, 63.



**Figure 1.** Predicted lower-energy conformers for 4(*S*)-hydroxyproline and relative energies in wavenumbers (configuration 1, N···H-O; configuration 2, N-H···O=C; configuration 3, N-H···O-H; a = *C<sub>γ</sub>-endo*; b = *C<sub>γ</sub>-exo*; subscripts denote different orientations of the 4-hydroxy group).



**Figure 2.** Predicted lower-energy conformers for 4(*R*)-hydroxyproline and relative energies in wavenumbers (configuration 1, N···H-O; configuration 2, N-H···O=C; configuration 3, N-H···O-H; a = *C<sub>γ</sub>-endo*; b = *C<sub>γ</sub>-exo*; subscripts denote different orientations of the 4-hydroxy group).

sample is vaporized with the green pulses (532 nm, ~10 ns) from a Q-switched Nd:YAG laser. The laser light is focused to obtain laser irradiances of ca.  $10^8$  W cm<sup>-2</sup>. The ablation products are diluted in the carrier gas which expands inside the Fabry–Pérot resonator forming a supersonic jet, where they are probed with the Fourier transform microwave spectrometer. Neon with stagnation pressures of ca. 5 bar was used as the carrier gas. Microwave excitation pulses of 0.2–0.4 μs (1–15 mW) polarized the molecules in the expanding jet. The subsequent free-induction-decay was recorded in the time-domain with 4k or 8k data points at 40 ns intervals, equivalent in the best case to a

frequency resolution of ca. 3 kHz. Due to the collinear arrangement of the jet and resonator axis, each rotational transition is split in two Doppler components so the resonance frequencies are taken as the arithmetic mean of both components. Frequency accuracy of the rotational transitions is below 3 kHz.

**Ab Initio Calculations.** The combination of spectroscopic data with ab initio quantum chemical calculations constitutes a powerful tool in the conformational analysis of biomolecules in gas phase.<sup>1</sup> Thus, the analysis of the microwave spectrum was guided by theoretical predictions of relevant molecular properties, such as rotational constants,

**Table 1.** Ab Initio Molecular Properties of the Lower-Energy Conformers of 4(*S*)-Hydroxyproline (MP2/6-311++G(d,p) basis set)

conformers	Relative Energies <sup>a</sup>	Rotational Constants			Electric Dipole Moment <sup>b</sup>				Nuclear Quadrupole Coupling Constants		
	$\Delta E$ (cm <sup>-1</sup> )	A (MHz)	B (MHz)	C (MHz)	$\mu_a$ (D)	$\mu_b$ (D)	$\mu_c$ (D)	$\mu_T$ (D)	$\chi_{aa}$ (MHz)	$\chi_{bb}$ (MHz)	$\chi_{cc}$ (MHz)
1a <sub>1</sub>	0.0	2575	1435	1329	5.0	1.3	2.8	5.9	1.16	1.70	-2.86
1a <sub>2</sub>	501.8	2633	1332	1283	3.9	3.9	2.0	5.9	1.74	1.92	-3.66
1a <sub>3</sub>	441.5	2617	1388	1302	6.4	2.8	1.5	7.1	1.50	1.86	-3.36
1b <sub>1</sub>	917.8	3346	1049	900	4.3	1.1	2.9	5.3	0.05	0.74	-0.79
1b <sub>2</sub>	1020.0	3287	1058	905	3.3	2.5	1.4	4.3	-0.28	0.71	-0.43
2a <sub>1</sub>	688.6	2526	1468	1321	0.3	2.5	1.5	3.0	2.09	-0.71	-1.38
2a <sub>2</sub>	409.9	2806	1288	1216	1.1	1.1	1.3	2.1	3.05	-4.84	1.79
2a <sub>3</sub>	590.5	2568	1443	1329	1.3	1.0	0.0	1.7	2.34	-0.98	-1.38
2b <sub>1</sub>	1227.3	3053	1108	968	0.4	0.0	0.5	0.7	1.42	2.25	-3.67
2b <sub>2</sub>	1358.3	3103	1087	954	1.6	0.9	0.6	2.0	1.53	2.33	-3.86
3a <sub>1</sub>	824.5	2522	1496	1330	0.6	0.4	0.1	0.8	2.01	-0.67	-1.33
3a <sub>2</sub>	1109.9	2865	1278	1176	0.3	0.3	0.0	0.4	2.88	-4.37	1.49
3a <sub>3</sub>	1183.4	2541	1458	1340	0.7	0.4	0.0	0.8	2.16	-0.51	-1.65

<sup>a</sup> Uncorrected for zero-point vibrational energies. <sup>b</sup> Where 1 D  $\approx 3.3356 \times 10^{-30}$  C m.

**Table 2.** Ab Initio Molecular Properties of the Lower-Energy Conformers of 4(*R*)-Hydroxyproline (MP2/6-311++G(d,p) basis set)

conformers	Relative Energies <sup>a</sup>	Rotational Constants			Electric Dipole Moment <sup>b</sup>				Nuclear Quadrupole Coupling Constants		
	$\Delta E$ (cm <sup>-1</sup> )	A (MHz)	B (MHz)	C (MHz)	$\mu_a$ (D)	$\mu_b$ (D)	$\mu_c$ (D)	$\mu_T$ (D)	$\chi_{aa}$ (MHz)	$\chi_{bb}$ (MHz)	$\chi_{cc}$ (MHz)
1a <sub>1</sub>	950.4	2995	1140	990	3.8	3.0	3.5	6.0	0.77	1.63	-2.39
1a <sub>2</sub>	791.4	2992	1148	992	4.0	3.4	0.9	5.4	0.69	1.54	-2.23
1b <sub>1</sub>	0.0	3490	1128	962	5.6	2.3	0.2	6.0	-1.21	-0.68	1.88
1b <sub>2</sub>	745.9	3442	1107	945	3.6	1.9	1.0	4.2	-1.14	-0.32	1.46
2a <sub>1</sub>	1352.3	2909	1154	1023	0.7	0.1	1.6	1.8	2.48	1.46	-3.93
2a <sub>2</sub>	1357.6	2999	1128	996	1.3	2.5	0.6	2.8	2.75	1.07	-3.82
2b <sub>1</sub>	985.1	3394	1107	942	0.7	0.0	1.6	1.8	2.12	2.65	-4.76
2b <sub>2</sub>	182.5	3548	1114	966	2.0	0.3	0.5	2.1	-0.79	2.39	-1.60
3a <sub>1</sub>	1889.4	2858	1191	1066	0.4	1.7	0.1	1.8	1.17	2.42	-3.59
3a <sub>2</sub>	1839.6	2857	1200	1055	1.0	0.1	1.3	1.6	1.29	2.26	-3.55
3b <sub>1</sub>	1723.2	3391	1140	1012	1.1	1.1	1.8	2.4	-1.06	1.95	-0.89
3b <sub>2</sub>	780.4	3562	1120	973	2.1	1.7	0.3	2.7	-1.53	2.39	-0.86

<sup>a</sup> Uncorrected for zero-point vibrational energies. <sup>b</sup> Where 1 D  $\approx 3.3356 \times 10^{-30}$  C m.

electric dipole moment components, and <sup>14</sup>N nuclear quadrupole coupling parameters for the most stable conformers. In this synergic strategy, theory helps with the interpretation of the spectra and at the same time, the experimental data provide a firm benchmark for theory. Starting with the same considerations previously used for proline,<sup>9</sup> we explored the different conformers of the two molecules. In this way, we combined the three plausible intramolecular hydrogen bonds for the  $\alpha$ -amino acid skeleton (configurations 1, N $\cdots$ H-O; 2, N-H $\cdots$ O=C; or 3, N-H $\cdots$ O-H) with *C<sub>\gamma</sub>-endo* (a) or *-exo* (b) bent ring conformations. The resulting six initial possibilities for each molecule were labeled as in proline (1a, 1b, 2a, 2b, 3a, and 3b). Additionally, we considered different orientations of the 4(*S*)- or 4(*R*)-hydroxy group, which could give rise to additional intramolecular hydrogen bonds within the molecule. The orientations of the 4-hydroxy group were labeled with subscripts on the conformer type. Quantum chemical calculations were done in two steps. A computationally effective B3LYP density functional model with a standard 6-31G(d,p) basis set was used for a preliminary full geometry optimization, which was refined using second-order Møller–Plesset perturbation theory and a larger 6-311++G(d,p) basis set.<sup>24</sup> This level of theory behaved satisfactorily in our previous studies of valine<sup>10</sup> and alanine.<sup>11</sup> The predicted lower-energy structures generated by the conformer search for 4(*S*)-hydroxy-

proline and 4(*R*)-hydroxyproline are shown in Figures 1 and 2. The calculated molecular properties are presented in Tables 1 and 2.

Since our experiment is conducted on a cooled supersonic jet, we anticipated that not all of the predicted conformers would be observable due to the crucial role of conformational relaxation.<sup>1,11,25</sup> While the ab initio calculations may predict a large number of in vacuo conformational minima, the kinetic control exerted by the supersonic expansion (dependent on conformational energy differences, barrier heights, and carrier gas) determines which minima relax to lower-energy forms and how many species finally prevail in the jet.

### Analysis of the Rotational Spectra

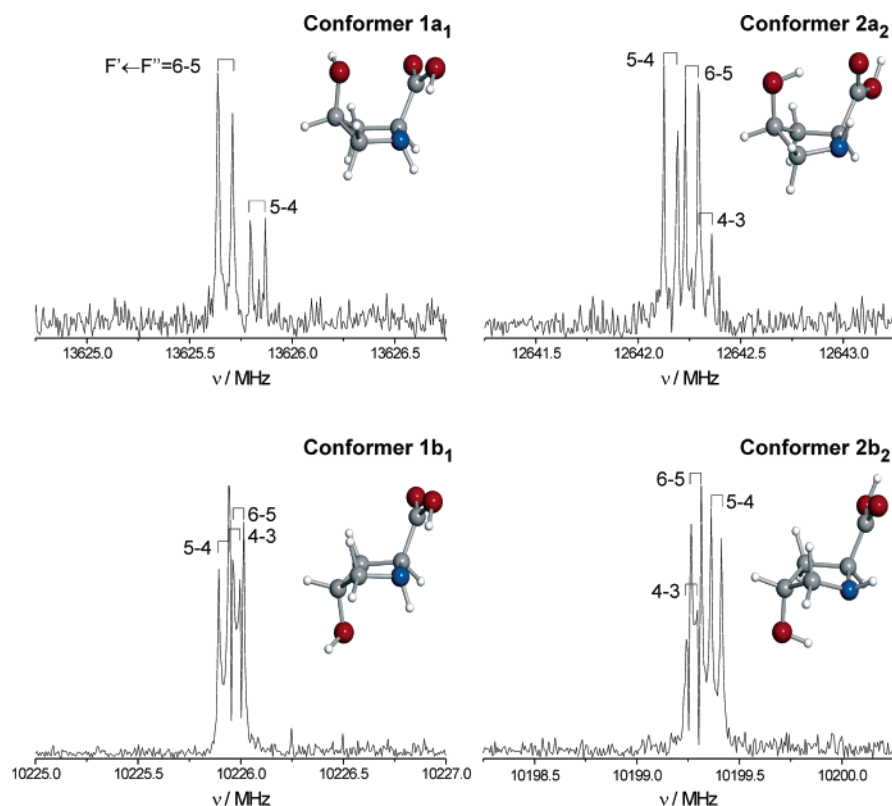
**4(*S*)-Hydroxyproline.** A frequency scan in the region of 8 GHz around the predictions for the most prominent  $\mu_a$ -type R-branch rotational transitions of the lower-energy conformers soon revealed a set of transitions which led to the assignment of a first conformer of the molecule. These measurements were extended to other  $\mu_b$ - and  $\mu_c$ -type R-branch transitions of this species. The presence of a small hyperfine effect in each transition (Figure 3), attributable to the nuclear quadrupole coupling interaction associated with the presence of a <sup>14</sup>N nucleus ( $I = 1$ ), confirmed that the carrier of the spectrum was a molecule containing a single <sup>14</sup>N atom. Rotational parameters were derived<sup>26</sup> using a Watson's semirigid rotor Hamiltonian<sup>27</sup>

(24) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian* 98, revision A.7; Gaussian, Inc.: Pittsburgh, PA, 1998.

(25) Ruoff, R. S.; Klots, T. D.; Emilsson, T.; Gutowsky, H. S. *J. Chem. Phys.* **1990**, *93*, 3142. (b) Godfrey, P. D.; Brown, R. D.; Rodgers, F. M. *J. Mol. Struct.* **1996**, *376*, 65. (c) Godfrey, P. D.; Brown, R. D. *J. Am. Chem. Soc.* **1998**, *120*, 10724.

(26) Pickett, H. M. *J. Mol. Spectrosc.* **1991**, *148*, 371.

(27) Watson, J. K. In *Vibrational Spectra and Structure*; Durig, J. R., Ed.; Elsevier: Amsterdam, 1977; Vol. 6, pp 1–89.



**Figure 3.**  $J_{K-1,K+1} = 5_{0,5} \leftarrow 4_{0,4}$  rotational transition for the observed conformers of 4(*S*)- and 4(*R*)-hydroxyproline, split into several hyperfine components by the nuclear quadrupole coupling effect created by the  $^{14}\text{N}$  nucleus ( $I = 1$ ). The hyperfine components are labeled with quantum numbers  $F = I + J$ . Each component appears as a doublet due to the Doppler effect (see Experimental and Theoretical Methods). The number of averaging cycles for these spectra was 100–400.

**Table 3.** Rotational Parameters of 4(*S*)-Hydroxyproline

	Conformer 1a <sub>1</sub>				Conformer 2a <sub>2</sub>
	parent	COOD	OD	ND	parent
$A$ (MHz) <sup>a</sup>	2556.87476(157) <sup>f</sup>	2518.17(40)	2494.392(300)	2484.993(308)	2756.16235(62)
$B$ (MHz)	1437.29323(78)	1430.2341(47)	1432.8709(46)	1433.9302(37)	1296.32013(148)
$C$ (MHz)	1320.17574(73)	1310.4593(39)	1306.0804(34)	1300.65107(292)	1238.76199(146)
$\Delta_J$ (kHz) <sup>b</sup>	0.5754(110)	[0.5754] <sup>g</sup>	[0.5754]	[0.5754]	0.4513(78)
$\Delta_{JK}$ (kHz)	−0.882(56)	[−0.882]	[−0.882]	[−0.882]	−0.844(52)
$\Delta_K$ (kHz)	1.29(33)	[1.29]	[1.29]	[1.29]	2.172(61)
$\delta_J$ (kHz)	0.1267(56)	[0.1267]	[0.1267]	[0.1267]	−0.02537(281)
$\delta_K$ (kHz)	1.27(33)	[1.27]	[1.27]	[1.27]	1.86(71)
$\chi_{aa}$ (MHz) <sup>c</sup>	1.0775(42)	0.99(26)	1.05(25)	1.11(29)	2.9941(61)
$\chi_{bb}$ (MHz)	1.6593(24)	1.53(44)	1.79(40)	1.70(51)	−5.0479(34)
$\chi_{cc}$ (MHz)	−2.7368(30)	−2.52(105)	−2.85(96)	−2.80(123)	2.0539(39)
$\sigma^d$ (kHz)	1.9	2.3	4.0	18.1	1.8
$N^e$	91	12	14	14	126

<sup>a</sup>  $A$ ,  $B$ , and  $C$  represent the rotational constants. <sup>b</sup>  $\Delta_J$ ,  $\Delta_{JK}$ ,  $\Delta_K$ ,  $\delta_J$ , and  $\delta_K$  are the quartic centrifugal distortion constants. <sup>c</sup>  $\chi_{\alpha\beta}$  ( $\alpha, \beta = a, b, \text{ or } c$ ) values are  $^{14}\text{N}$  nuclear quadrupole coupling parameters. <sup>d</sup> Root-mean-square deviation of the fit. <sup>e</sup> Number of fitted transitions. <sup>f</sup> Standard error in parentheses in units of the last digit. <sup>g</sup> Parameters in square brackets were kept fixed in the fit.

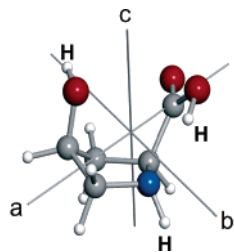
(asymmetric reduction) supplemented with nuclear quadrupole coupling terms.<sup>28</sup> Inspection of the experimental rotational constants (Table 3) revealed a very good agreement with the predictions for the 1a<sub>1</sub> conformer. Moreover, the microwave power needed for optimum polarization of the rotational transitions was consistent with the predicted magnitudes for the components of the electric dipole moment. A definitive assessment of the presence of conformer 1a<sub>1</sub> in the jet was obtained from the excellent agreement between the experimental and predicted values for the  $^{14}\text{N}$  nuclear quadrupole coupling

constants. The dependence of the nuclear quadrupole coupling parameters with the molecular electric field gradient at the position of the  $^{14}\text{N}$  atom and its orientation with respect to the principal inertial axis system make these parameters very sensitive to small structural changes. In consequence, they serve as a conclusive tool to discriminate between the different conformers.<sup>10,11</sup> In particular, these parameters excluded the 1a<sub>2</sub> and 1a<sub>3</sub> conformers, which mainly differ in the orientation of the 4-hydroxyl group.

A further confirmation of the identity of this first conformer was determined through the analysis of the rotational spectrum of a deuterated sample of 4(*S*)-hydroxyproline. The isotopic

(28) Gordy, W.; Cook, R. L. *Microwave Molecular Spectra*; Wiley: New York, 1984.

**Table 4.** Situation of the Hydrogen Atoms of the Functional Groups in Conformer 1a<sub>1</sub> of 4(*S*)-Hydroxyproline, Together with the Derived Principal Axis Coordinates for the Substituted Atoms



atom	Conformer 1a <sub>1</sub>		
	a	b	c
H (–COOH)	–0.869(17) <sup>a</sup>	+1.421(11)	+1.019(15)
H (–OH)	+0.36(4)	–1.971(8)	+1.038(14)
H (–NH)	+0.64(2)	+2.296(7)	–0.68(2)

<sup>a</sup> Errors in parentheses, in units of the last digit, calculated according to Costain.<sup>30</sup>

shifts calculated from the predicted geometry of conformer 1a<sub>1</sub> were used to search for the rotational spectrum of the three monodeuterated species, –COOD, –OD, and –ND, which were found close to the predictions. The rotational transitions of the deuterated species were analyzed as described for the parent species. The nuclear quadrupole coupling effects caused by the presence of the D atom ( $I = 1$ ) were not resolved and were thus not considered. The derived rotational parameters for the deuterated isotopomers are shown also in Table 3.

The inertial data collected for conformer 1a<sub>1</sub>, limited to 12 moments of inertia from only four isotopomers, are clearly insufficient to derive the full structure of this molecule, with 18 atoms and no symmetry. However, some structural information on the positions of the deuterated hydrogen atoms can be obtained using the substitution method of Kraitchman.<sup>29</sup> The substitution coordinates of the three deuterated hydrogen atoms are shown in Table 4. The sign of the different coordinates was inferred by comparison with the ab initio structures, and the errors quoted were calculated as  $\delta z_i = 0.0012/|z_i|$  Å, according to Costain.<sup>30</sup> Even when we consider the uncertainties associated with the large change of mass on deuterium substitution, this structural information definitively supports the assignment of the observed conformer through the location of the hydrogen atoms, in particular, the orientation of the 4-hydroxy group.

Once the presence of the global minimum 1a<sub>1</sub> was unambiguously confirmed, we investigated the existence of other conformers in the jet. A set of weaker  $\mu_a$ -type R-branch rotational transitions around 7.5 GHz split by <sup>14</sup>N nuclear quadrupole hyperfine coupling led to the assignment of a second conformer, analyzed with the same Hamiltonian described above for conformer 1a<sub>1</sub>. The derived rotational parameters for the second conformer are collected in Table 3. A first inspection of the experimental rotational constants suggested that the newly observed species could be identified as conformer 2a<sub>2</sub> (N–H···O=C intramolecular hydrogen bond, C<sub>γ</sub>-endo), but an unequivocal identification of this conformer resulted again from the excellent agreement between the experimental and predicted

(Table 1) nuclear quadrupole coupling constants, which excluded other 2a conformers. For conformer 2a<sub>2</sub>, it was not possible to observe the spectrum of minor isotopomers due to the reduced intensity of the rotational transitions. After long frequency scans, no more conformers could be detected in the supersonic expansion for 4(*S*)-hydroxyproline.

**4(*R*)-Hydroxyproline.** The analysis of 4(*R*)-hydroxyproline proceeded in a similar way. Several scans around 10 GHz led to the assignment of a first conformer of the molecule, with the presence in all transitions of the characteristic hyperfine components due to the nuclear quadrupole coupling interaction caused by a <sup>14</sup>N atom (Figure 3). Unequivocal identification of the observed rotamer as conformer 1b<sub>1</sub> resulted again from the very good agreement between the predicted (Table 2) and experimental (Table 5) rotational constants and the excellent match of the <sup>14</sup>N nuclear quadrupole coupling parameters, which particularly excluded the alternative 1b<sub>2</sub> conformer. The analysis of minor isotopomers of this species further proved the presence in the jet of conformer 1b<sub>1</sub>. The rotational spectrum of the monodeuterated –COOD and –ND species was assigned from a sample of deuterated 4(*R*)-hydroxyproline. Additionally, it was possible to observe with difficulty four transitions of the <sup>13</sup>C<sub>α</sub> monosubstituted species in natural abundance (ca. 1%). The coordinates for the substituted atoms derived from Kraitchman equations<sup>29</sup> are shown in Table 6 and support unambiguously the assignment of conformer 1b<sub>1</sub>.

The search for additional conformers of 4(*R*)-hydroxyproline was then continued. New scans revealed the presence of a second conformer in the jet, which was analyzed as described before. The consideration of all the experimental information available, in particular, the analysis of the nuclear quadrupole coupling interaction caused by the <sup>14</sup>N nucleus, led to the identification of conformer 2b<sub>2</sub> (rotational parameters in Table 5). The weaker intensity of this spectrum precluded the observation of minor isotopomers. No more conformers could be detected in the supersonic expansion for 4(*R*)-hydroxyproline. All experimental rotational transitions collected in this work are given as Supporting Information.

## Discussion

Two conformers for each of 4(*S*)-hydroxyproline and 4(*R*)-hydroxyproline have been observed for the first time in a supersonic expansion. The reduced number of observed conformers compared to the number of predicted low-energy minima can be rationalized considering the collisional relaxation in the jet of high-energy forms to the most stable conformers through low-energy barriers.<sup>1,11,25</sup> In the hydroxyprolines, conformational relaxation is likely to occur within those rotamers differing only in the orientation of the 4-hydroxy group. Moreover, a plausible configuration 3 (N–H···O–H) was observed neither in proline<sup>9</sup> nor in the two hydroxyprolines studied here. This fact is consistent with the nonobservation of this configuration in glycine,<sup>5c</sup> alanine,<sup>11</sup> and valine<sup>10</sup> and suggests that the near 180° rotation of the whole carboxyl group required to interconvert configuration 3 into configuration 2 proceeds analogously through an affordable energy barrier.<sup>25b</sup> Collisional relaxation ensures that the observed conformers in the jet are the most stable.

Our gas-phase study of proline<sup>9</sup> established that configuration 1 (N···H–O intramolecular hydrogen bond) was giving rise to

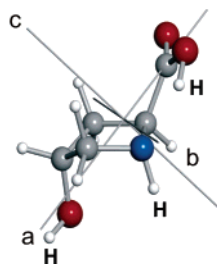
(29) Kraitchman, J. *Am. J. Phys.* **1953**, *21*, 17.

(30) Costain, C. C. *Trans. Am. Crystallogr. Assoc.* **1966**, *2*, 157. (b) van Eijck, B. P. *J. Mol. Spectrosc.* **1982**, *91*, 348. (c) Demaison, J.; Rudolph, H. D. *J. Mol. Spectrosc.* **2002**, *215*, 78.

**Table 5.** Rotational Parameters of 4(*R*)-Hydroxyproline

	Conformer 1b <sub>1</sub>				Conformer 2b <sub>2</sub>
	parent	<sup>13</sup> C <sub>α</sub>	COOD	ND	parent
A (MHz) <sup>a</sup>	3502.01632(75) <sup>f</sup>	3492.697(64)	3437.340(274)	3417.820(259)	3548.8528 (90)
B (MHz)	1120.744302(140)	1119.67725(58)	1115.05937(226)	1115.12418(221)	1107.26009 (25)
C (MHz)	956.214925(114)	956.01660(57)	947.24696(188)	951.79367(187)	958.29775 (25)
Δ <sub>J</sub> (kHz) <sup>b</sup>	0.08553 (68)	[0.08553]	[0.08553]	[0.08553]	0.08456 (79)
Δ <sub>JK</sub> (kHz)	0.8091 (80)	[0.8091]	[0.8091]	[0.8091]	0.3524 (35)
Δ <sub>K</sub> (kHz)	[0.0] <sup>g</sup>	[0.0]	[0.0]	[0.0]	[0.0]
δ <sub>J</sub> (kHz)	0.00723(63)	[0.00723]	[0.00723]	[0.00723]	7.48 (1.16)
δ <sub>K</sub> (kHz)	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]
χ <sub>aa</sub> (MHz) <sup>c</sup>	-1.1518(44)	[-1.1518]	-1.360(123)	-1.227(125)	-0.6797 (47)
χ <sub>bb</sub> (MHz)	-0.6057(79)	[-0.6057]	-0.356(108)	-0.557(107)	2.387 (22)
χ <sub>cc</sub> (MHz)	1.7575(79)	[1.7575]	1.716(108)	1.803(107)	-1.707 (22)
σ <sup>d</sup> (kHz)	2.4	3.5	3.4	3.2	2.6
N <sup>e</sup>	94	4	17	17	98

<sup>a</sup> A, B, and C represent the rotational constants. <sup>b</sup> Δ<sub>J</sub>, Δ<sub>JK</sub>, Δ<sub>K</sub>, δ<sub>J</sub>, and δ<sub>K</sub> are the quartic centrifugal distortion constants. <sup>c</sup> χ<sub>αβ</sub> (α, β = a, b, or c) values are <sup>14</sup>N nuclear quadrupole coupling parameters. <sup>d</sup> Root-mean-square deviation of the fit. <sup>e</sup> Number of fitted transitions. <sup>f</sup> Standard error in parentheses in units of the last digit. <sup>g</sup> Parameters in square brackets were kept fixed in the fit.

**Table 6.** Situation of the Hydrogen Atoms of the Functional Groups in Conformer 1b<sub>1</sub> of 4(*R*)-Hydroxyproline, Together with the Derived Principal Axis Coordinates for the Substituted Atoms

atom	Conformer 1b <sub>1</sub>		
	a	b	c
C <sub>α</sub>	-0.28(5) <sup>a</sup>	-0.18(8)	-0.59(3)
H (-COOH)	-1.509(10)	+1.653(9)	-0.1(2)
H (-NH)	+0.76(2)	+1.354(11)	-1.316(11)

<sup>a</sup> Errors in parentheses, in units of the last digit, calculated according to Costain.<sup>30</sup>

the two most stable conformers, which only differed in their ring conformation, a bent C<sub>γ</sub>-endo for the most stable conformer or a C<sub>γ</sub>-exo arrangement. The most stable conformer in both 4(*S*)-hydroxyproline and 4(*R*)-hydroxyproline exhibits configuration 1 (N···H—O), like in proline, while the second stable conformer presents configuration 2 (N—H···O=C), not observed in proline.<sup>9</sup> The reversal of the usual stability trend with respect to the simpler aliphatic amino acids, already observed for proline, can be understood considering that the formation of a pyrrolidine ring implies losing an amino H atom capable of type 2 (N—H···O=C) intramolecular hydrogen bonding. The two observed conformers of 4(*S*)-hydroxyproline present a similar *endo* ring conformation, while in the case of 4(*R*)-hydroxyproline, this preference is reversed and its two conformers exhibit an *exo* ring conformation. In consequence, the most obvious effect of the introduction of the 4-hydroxy group is to favor a particular *endo* or *exo* form of the pyrrolidine ring in each molecule.

The observed conformational preferences of 4(*S*)- and 4(*R*)-hydroxyproline can be attributed to different contributions. A first factor to consider is intramolecular hydrogen bonding established by the 4-hydroxy group. Hydrogen bonds C<sub>γ</sub>—O···H—N (1b<sub>1</sub>) and C<sub>γ</sub>—O—H···N (2b<sub>2</sub>) are apparent in the two

observed conformers of 4(*R*)-hydroxyproline, supported by both directionality and predicted hydrogen bond distances (*r*<sub>O···H</sub> = 2.71 Å and *r*<sub>H···N</sub> = 2.32 Å). These interactions are expected to produce a σ-bond cooperativity<sup>31</sup> with the intramolecular hydrogen bonds established between the carboxylic and amino moieties, enhancing the stability of those conformers. In conformer 2a<sub>2</sub> of 4(*S*)-hydroxyproline, there is also evidence of the presence of a second intramolecular hydrogen bond, C<sub>γ</sub>—O—H···O=C (*r*<sub>O···H</sub> = 2.18 Å), that competes with the N—H···O=C interaction (*r*<sub>O···H</sub> = 2.31 Å), as suggested from the rotation of the carboxylic group to establish a bifurcated linkage to both C<sub>γ</sub>—O—H and N—H groups. However, no additional intramolecular hydrogen bond is apparent in conformer 1a<sub>1</sub> of 4(*S*)-hydroxyproline, where the hydrogen atom of the 4-hydroxy group adopts a staggered arrangement which points out of the ring. It should be emphasized that the experimental coordinates for this hydrogen atom derived from the OH(D) isotopic substitution (Table 4) agree very satisfactorily with the predicted coordinates and support the ab initio geometry. Considering that conformer 1a<sub>1</sub> is predicted to be the global minimum of 4(*S*)-hydroxyproline, we must admit that its stabilization should be originated by another factor different than intramolecular hydrogen bonding. The arrangement of the 4-hydroxy group with respect to the carbonyl group suggests a n-π\* interaction, which arises from the hyperconjugative delocalization of a nonbonding electron pair (n) from the 4-hydroxyl oxygen to the π\* orbital at the carboxylic group carbon, reminiscent of the Bürgi–Dunitz trajectory of organic chemistry.<sup>19d,32</sup> A meaningful n-π\* interaction has been defined as one in which the O···C=O distance is δ<sub>BD</sub> ≤ 3.2 Å and the ∠O···C=O angle is 99° < τ<sub>BD</sub> < 119°.<sup>32c</sup> For the 2a<sub>1</sub> conformer of 4(*S*)-hydroxyproline, these parameters are calculated to be δ<sub>BD</sub> = 2.97 Å and τ<sub>BD</sub> < 108.5°, optimal for such an interaction to occur.

In this context, the possibility of further stereoelectronic contributions to stabilize the preferred ring-puckering conformation can also be considered. The so-called gauche effect<sup>19</sup> would favor a gauche arrangement of the O—C<sub>γ</sub>—C<sub>δ</sub>—N chain of the pyrrolidine ring. In consequence, the *endo* conformation in 4(*S*)-hydroxyproline and the *exo* conformation in 4(*R*)-hydroxyproline

(31) Steiner, T. *Angew. Chem., Int. Ed.* **2002**, *41*, 48.

(32) Bürgi, H. B.; Dunitz, J. D.; Shefter, E. *J. Am. Chem. Soc.* **1973**, *95*, 5065. (b) Bürgi, H. B.; Dunitz, J. D.; Lehn, J. M.; Wipff, G. *Tetrahedron* **1974**, *30*, 1563. (c) Hinderaker, M. P.; Raines, R. T. *Protein Sci.* **2003**, *12*, 1188.



would be benefited instead of the alternative *exo* (4(*S*)-hydroxyproline) and *endo* (4(*R*)-hydroxyproline) conformations, where the O–C<sub>γ</sub>–C<sub>δ</sub>–N chain adopts an *anti* arrangement. Our experimental findings, which constitute the first experimental evidence of the intrinsic puckering preferences of the 4-hydroxyprolines in gas phase, are consistent with the *gauche* empirical rule, but we must be aware that the observed conformational preferences of the 4-hydroxyprolines may result from a contribution of different covalent and noncovalent interactions in which intramolecular hydrogen bonds play a relevant role.

Care should be taken also to extrapolate the experimental results from the isolated molecule observed in the collisionless environment of the supersonic jet to the condensed media in which biology takes place, characterized by strong intermolecular interactions. Despite these preventions, our results in gas phase indicate for the pyrrolidine ring puckering, the same propensity observed in collagen-like peptides. The most recent models of collagen, based on different synthetic (X–Y–glycine) tripeptide mimics,<sup>18a,20a,b,21</sup> remarkably the crystalline structure of the triple helical (proline–proline–glycine)<sub>10</sub> (PPG<sub>10</sub>) peptide,<sup>20b</sup> clearly indicate a strong correlation between the position of the imino acid in the chain and its ring conformation. Theoretical<sup>20c,d</sup> and experimental<sup>20a,b</sup> studies on PPG<sub>10</sub> confirm an alternation of *endo* and *exo* puckerings for proline in the X and Y positions of the chain to give peptide dihedrals, which would result in a tight packing of the triple helix. The propensity of 4(*R*)-hydroxyproline to *exo* puckering conformations, now confirmed in gas phase, would thus represent a form of

preorganization of the triple helix. Conversely, the tendency of 4(*S*)-hydroxyproline to *endo* puckerings could favor the triple helix stability if placed in X position. However, this effect has not been observed in collagen,<sup>17b</sup> a fact attributed to steric repulsions of the 4-hydroxy group with the adjacent chain.<sup>20a</sup> Recent experiments with other peptide mimics<sup>19,21,33,34</sup> and unusual collagen structures<sup>35</sup> suggest that the formation of the triple helix is originated by a delicate balance of different intraresidue and interchain interactions not yet untangled. This should remind us that the problem of collagen stability is an area of active research far from being completely solved. In this sense, the study of amino acids and small peptides under isolation conditions in gas phase can help to understand the structural bases of larger molecules which support life.

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**Supporting Information Available:** Tables of experimental rotational frequencies of all the observed isotopic species of 4(*S*)- and 4(*R*)-hydroxyproline. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JA045955M

- (33) Doi, M.; Nishi, Y.; Uchiyama, S.; Nishiuchi, Y.; Nakazawa, T.; Ohkubo, T.; Kobayashi, Y. *J. Am. Chem. Soc.* **2003**, *125*, 9922.  
(34) Barth, D.; Milbradt, A. G.; Renner, C.; Moroder, L. *ChemBioChem* **2004**, *5*, 79.  
(35) Bann, J. G.; Bächinger, H. P. *J. Biol. Chem.* **2000**, *275*, 24466. (b) Mizuno, K.; Hayashi, T.; Bächinger, H. P. *J. Biol. Chem.* **2003**, *278*, 32373.