Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting information for:

A rotational study of the AlaAla dipeptide

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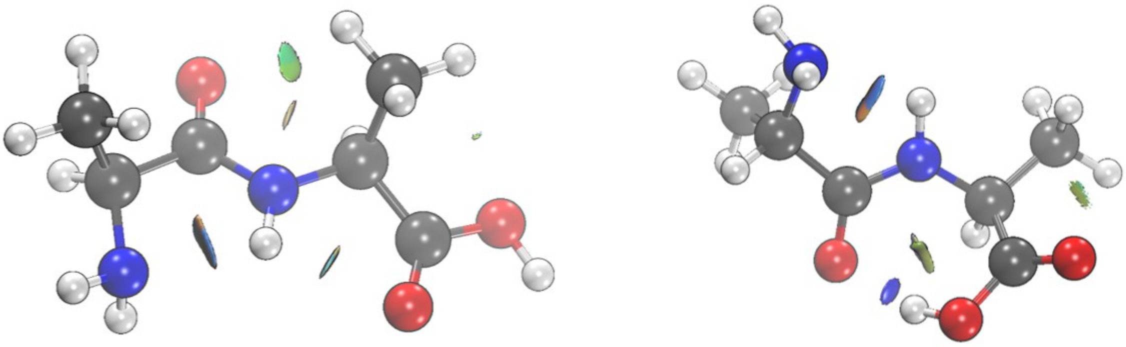
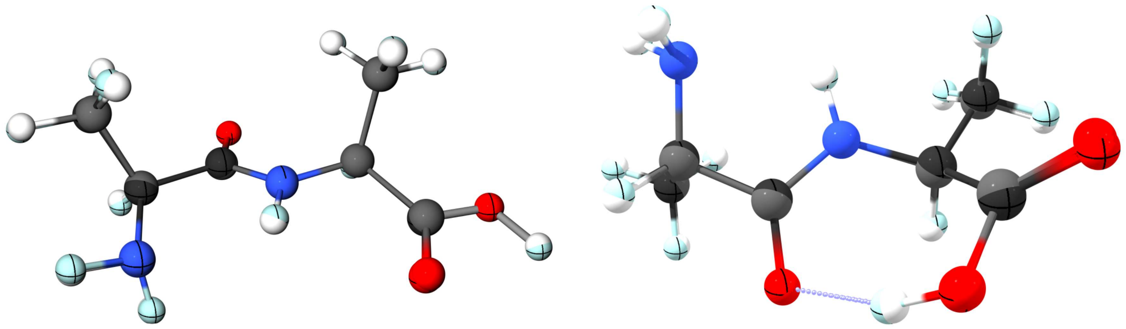
Fig. S1. Detected conformers of AlaAla with the analysis of the interactions using NCIplot.[1,2] Red surfaces correspond to strong repulsion forces, blue surfaces to strong attraction forces and green surfaces to weak attractive interactions.



cis-I

trans-I

Fig. S2. Superimposed structures of the two detected conformers of AlaAla using MP2/6-311++G(d,p) and B3LYPD3/def2tvtz. The representation without marks and white colors for the hydrogens account for the MP2 method, while that with marked atoms and bluish hidrogens account for B3LYPD3. As can be seen, both structrures are very similar with minimal variations in the orientation of the methyl groups.



Cis-I **Trans-1**

Table S01. Measured frequencies and residuals (in MHz) for the rotational transitions of rotamer 1 of AlaAla dipeptide using the LA-CP-FTMW spectrometer. Note that, due to the nuclear quadrupole coupling, each line is split into several hyperfine components and only the center of frequencies was used.

J'	K'_a	K'_c	J''	K''_a	K''_c	$\nu_{obs.}$	$\nu_{obs.}-\nu_{cal.}$
4	0	4	3	1	3	3308.557	0.220
6	1	5	5	2	4	3368.321	-0.007
2	1	2	1	0	1	3571.837	-0.188
8	2	6	8	1	7	3589.758	-0.010
7	2	5	7	1	6	3611.890	0.174
6	2	4	6	1	5	3688.729	0.269
5	2	3	5	1	4	3802.035	-0.095
4	2	2	4	1	3	3933.318	-0.167
3	2	1	3	1	2	4063.612	-0.154
2	2	1	2	1	2	4434.403	-0.700
7	1	6	6	2	4	4498.509	0.072
5	0	5	4	1	4	4547.564	-0.217
3	2	2	3	1	3	4569.170	0.336
3	1	3	2	0	2	4577.003	0.355
4	2	3	4	1	4	4748.796	0.360
7	1	6	6	2	5	4766.996	-0.101
3	1	2	2	0	2	5101.967	0.297
5	2	3	5	1	5	5112.496	0.720
6	2	5	6	1	6	5248.235	0.006
4	1	4	3	0	3	5544.802	0.565
6	0	6	5	1	5	5785.481	-0.124
10	3	8	10	2	8	6062.326	-0.230
8	1	7	7	2	6	6187.481	-0.398
10	3	7	10	2	8	6248.386	0.336
9	2	8	9	1	9	6351.552	-0.321
4	1	3	3	0	3	6418.465	-0.198
5	1	5	4	0	4	6482.832	-0.127
2	2	1	1	1	0	6528.357	-0.404
2	2	0	1	1	0	6532.693	-0.070
2	2	1	1	1	1	6616.537	0.254
2	2	0	1	1	1	6620.536	0.251
8	3	5	8	2	6	6685.661	0.049
7	3	4	7	2	5	6856.920	0.453
5	3	2	5	2	3	7073.818	-0.045
4	3	1	4	2	2	7127.689	0.101
3	3	0	3	2	2	7175.592	-0.001
4	3	2	4	2	3	7186.335	0.072
4	3	1	4	2	3	7186.940	-0.124
5	3	3	5	2	4	7207.877	0.131
6	3	4	6	2	5	7244.807	0.148
7	3	5	7	2	6	7301.805	-0.310
8	3	6	8	2	7	7385.207	-0.143
6	1	6	5	0	5	7403.694	0.273
3	2	2	2	1	1	7575.561	-0.029
3	2	1	2	1	2	7858.231	0.119

Table S02. Measured frequencies and residuals (in MHz) for the rotational transitions of rotamer 1 of AlaAla dipeptide using the LA-CP-FTMW spectrometer. Note that, due to the nuclear quadrupole coupling, each line is split into several hyperfine components and only the center of frequencies was used.

J'	K'_a	K'_c	J''	K''_a	K''_c	$\nu_{obs.}$	$\nu_{obs.}-\nu_{cal.}$
4	1	4	3	1	3	4701.012	0.026
3	1	3	2	0	2	4778.583	-0.024
4	0	4	3	0	3	4825.104	-0.045
4	2	3	3	2	2	4852.879	0.025
5	0	5	4	1	4	4984.674	0.056
4	1	3	3	1	2	4997.261	-0.026
5	1	5	4	1	4	5871.043	-0.003
5	0	5	4	0	4	6009.766	0.002
5	4	2	4	4	1	6075.517	-0.252
5	3	3	4	3	2	6078.747	0.081
5	3	2	4	3	1	6080.382	0.088
9	3	6	9	2	7	6238.946	-0.052
5	1	4	4	1	3	6240.380	0.001
6	0	6	5	1	5	6295.342	0.041
8	3	5	8	2	6	6409.841	0.032
2	2	1	1	1	0	6466.299	0.002
2	2	0	1	1	1	6543.580	0.054
7	3	4	7	2	5	6547.023	-0.073
6	3	3	6	2	4	6648.647	-0.040
5	3	2	5	2	3	6717.173	0.024
4	3	1	4	2	2	6758.320	-0.053
3	3	1	3	2	2	6794.850	0.015
4	3	2	4	2	3	6803.165	0.119
5	3	3	5	2	4	6819.379	-0.059
6	3	4	6	2	5	6847.600	-0.091
8	1	7	7	2	6	6851.837	-0.109
7	3	5	7	2	6	6891.845	0.024
5	1	5	4	0	4	6896.214	0.022
8	3	6	8	2	7	6955.995	-0.016
6	1	6	5	1	5	7038.065	0.016
9	3	7	9	2	8	7044.437	0.024
10	3	8	10	2	9	7161.017	0.047
6	0	6	5	0	5	7181.722	-0.007
6	2	5	5	2	4	7269.169	-0.006
6	5	1	5	5	0	7290.376	-0.048
6	4	3	5	4	2	7293.140	0.008
6	3	4	5	3	3	7297.506	0.080
6	2	4	5	2	3	7370.240	0.028
6	1	5	5	1	4	7478.888	0.009
7	0	7	6	1	6	7598.319	0.016
3	2	2	2	1	1	7605.956	0.047
6	1	6	5	0	5	7924.471	-0.010

Table S03. Measured frequencies and residuals (in MHz) for the nuclear quadrupole coupling hyperfine components of rotamer 1 of AlaAla dipeptide using the LA-MB-FTMW spectrometer.

J'	K'_{-1}	K'_{+1}	J''	K''_{-1}	K''_{+1}	I'	F'	I''	F''	$\nu_{obs.}$	$\nu_{obs.}-\nu_{cal.}$
3	1	3	2	0	2	0	3	2	3	4575.302	-0.001
3	1	3	2	0	2	1	3	2	2	4575.795	0.002
3	1	3	2	0	2	0	3	0	2	4576.012	-0.002
3	1	3	2	0	2	2	5	2	4	4576.447	0.004
3	1	3	2	0	2	0	3	1	2	4576.663	-0.002
3	1	3	2	0	2	2	4	2	3	4576.873	0.002
3	1	3	2	0	2	2	3	2	2	4576.947	-0.001
3	1	3	2	0	2	2	2	2	1	4576.982	-0.001
3	1	3	2	0	2	1	4	1	3	4577.067	0.001
3	1	2	2	0	2	1	4	2	3	5101.288	0.006
3	1	2	2	0	2	2	4	1	3	5101.566	-0.001
3	1	2	2	0	2	2	5	2	4	5102.004	0.002
4	1	4	3	0	3	2	6	2	5	5544.045	0.006
4	1	4	3	0	3	2	5	2	4	5544.495	0.006
4	1	4	3	0	3	2	3	2	2	5544.535	-0.002
4	1	4	3	0	3	1	5	1	4	5544.600	0.001
4	1	4	3	0	3	2	4	2	3	5544.765	0.001
4	1	3	3	0	3	1	5	2	4	6418.317	0.001
4	1	3	3	0	3	2	3	1	2	6418.412	-0.004
4	1	3	3	0	3	2	5	1	4	6418.517	-0.003
4	1	3	3	0	3	2	6	2	5	6419.042	0.001
5	1	5	4	0	4	2	3	2	2	6482.617	0.001
5	1	5	4	0	4	0	5	0	4	6482.737	0.001
5	1	5	4	0	4	2	7	2	6	6482.787	-0.001
5	1	5	4	0	4	1	4	1	3	6483.092	-0.006
5	1	5	4	0	4	2	6	2	5	6483.217	-0.004
5	1	5	4	0	4	1	6	1	5	6483.282	-0.002
5	1	5	4	0	4	2	5	2	4	6483.577	0.001
2	2	1	1	1	0	1	1	1	1	6527.632	0.001
2	2	1	1	1	0	2	2	1	1	6527.967	-0.004
2	2	1	1	1	0	1	2	2	1	6528.037	-0.003
2	2	1	1	1	0	2	2	1	2	6528.157	0.003
2	2	1	1	1	0	2	1	2	1	6528.368	-0.003
2	2	1	1	1	0	0	2	1	1	6528.603	-0.001
2	2	1	1	1	0	0	2	1	1	6528.607	0.003
2	2	1	1	1	0	2	4	2	3	6528.687	-0.001
2	2	1	1	1	0	2	3	2	2	6528.893	-0.001
2	2	1	1	1	0	1	2	1	1	6529.248	0.003
2	2	1	1	1	0	1	2	1	1	6529.248	0.003
2	2	1	1	1	0	1	3	2	3	6529.348	0.001
2	2	1	1	1	0	1	3	2	3	6529.348	0.001
2	2	1	1	1	0	1	3	1	2	6529.457	0.002
2	2	1	1	1	0	0	2	0	1	6529.522	-0.006
2	2	1	1	1	0	1	2	2	2	6530.222	-0.004
2	2	1	1	1	0	2	1	1	0	6530.667	0.002

Table S04. Measured frequencies and residuals (in MHz) for the nuclear quadrupole coupling hyperfine components of rotamer 2 of AlaAla dipeptide using the LA-MB-FTMW spectrometer.

J'	K'_{-1}	K'_{+1}	J''	K''_{-1}	K''_{+1}	I'	F'	I''	F''	$\nu_{obs.}$	$\nu_{obs.}-\nu_{cal.}$
4	1	4	3	1	3	1	5	1	4	4700.946	-0.001
4	1	4	3	1	3	2	6	2	5	4701.005	0.001
4	1	4	3	1	3	1	4	1	3	4701.078	-0.004
4	1	4	3	1	3	2	5	2	4	4701.098	0.003
3	1	3	2	0	2	1	4	1	3	4778.407	0.002
3	1	3	2	0	2	2	5	2	4	4778.587	0.004
3	1	3	2	0	2	2	3	2	2	4778.702	0.001
3	1	3	2	0	2	2	4	2	3	4778.922	0.001
4	0	4	3	0	3	1	5	1	4	4825.138	0.006
4	0	4	3	0	3	2	4	2	3	4825.157	0.001
4	0	4	3	0	3	2	6	2	5	4825.172	0.001
4	0	4	3	0	3	1	4	1	3	4825.223	-0.001
4	0	4	3	0	3	2	5	2	4	4825.238	-0.001
4	1	4	3	0	3	1	5	1	4	5850.086	0.005
4	1	4	3	0	3	2	6	2	5	5850.276	0.001
4	1	4	3	0	3	0	4	0	3	5850.296	-0.004
4	1	4	3	0	3	2	4	2	3	5850.436	0.001
4	1	4	3	0	3	2	5	2	4	5850.641	0.002
4	1	4	3	0	3	1	3	1	2	5850.661	-0.001
5	1	5	4	1	4	1	6	1	5	5871.041	0.001
5	1	5	4	1	4	2	5	2	4	5871.061	-0.005
5	1	5	4	1	4	2	7	2	6	5871.073	-0.004
5	1	5	4	1	4	1	4	1	3	5871.119	-0.001
5	1	5	4	1	4	2	6	2	5	5871.134	-0.003
5	0	5	4	0	4	1	6	1	5	6009.751	-0.001
5	0	5	4	0	4	2	4	2	3	6009.776	0.007
5	0	5	4	0	4	2	7	2	6	6009.800	0.007
5	0	5	4	0	4	2	6	2	5	6009.859	-0.007
2	2	1	1	1	0	1	2	2	1	6465.547	-0.001
2	2	1	1	1	0	2	1	2	1	6465.702	-0.002
2	2	1	1	1	0	1	3	1	2	6466.277	-0.002
2	2	1	1	1	0	2	4	2	3	6466.302	0.003
2	2	1	1	1	0	2	3	2	2	6466.402	-0.002
2	2	1	1	1	0	2	3	2	2	6466.402	-0.002
2	2	1	1	1	0	1	1	1	1	6466.622	-0.003
2	2	1	1	1	0	1	2	2	2	6467.247	-0.004
2	2	0	1	1	1	2	3	2	2	6542.922	0.003
2	2	0	1	1	1	2	3	2	3	6543.082	-0.001
2	2	0	1	1	1	2	2	1	2	6543.232	0.001
2	2	0	1	1	1	2	4	2	3	6543.592	-0.002
2	2	0	1	1	1	1	2	2	2	6543.807	0.002
2	2	0	1	1	1	1	3	1	2	6543.967	0.002
2	2	0	1	1	1	1	2	2	1	6544.267	0.002
2	2	0	1	1	1	2	1	2	1	6544.432	0.003
5	1	5	4	0	4	2	4	2	3	6895.947	-0.003
5	1	5	4	0	4	1	6	1	5	6895.987	-0.003
5	1	5	4	0	4	1	5	1	4	6896.147	0.004
5	1	5	4	0	4	2	5	2	4	6896.346	0.001

5	1	5	4	0	4	2	6	2	5	6896.537	-0.001

Calculation of the relative population from the relative intensities measurements.

In order to estimate the relative populations the following procedure was followed:

1 – Selected transitions were identified. Whenever it was possible the same transitions were used for the different conformers.
2 – All the measurements were done in the narrowband cavity and the microwave polarization power was adjusted for each individual transition (dipole moment) to ensure a linear fast passage (LFP) regime condition so to avoid multi-resonance effects.[3,4]
3 – The area under each of the hyperfine components was calculated and the total area was summed to get a number for the total intensity of a selected rotational transition.
4 – To estimate the relative intensities we assumed a Boltzmann distribution. Furthermore, we assume that the cooling in the supersonic expansion brings all the molecular systems to the lowest vibrational state of each observed conformer. Thus, the energy difference (ΔE) between two conformers (1 and 2) in their lowest vibrational and rotational ground states can be obtained[5,6] using:

$$\Delta E = E_2 - E_1 = \ kT * \ln\left(\frac{I_1 \Delta v_1 g_2 \mu_{abc,2} \gamma_2 v_2^2}{I_2 \Delta v_1 g_1 \mu_{abc,1} \gamma_1 v_1^2}\right)$$

> where *T* is the temperature is the temperature prior to the expansion, *k* is the Boltzmann constant, *I* and *Δv* are the peak intensity and line width at half height which we measured as the area, *g* is the degeneracy, *μ* is the dipole moment component, *γ* is the line strength, and *v* is the transition frequency.

5 – Thus, measuring the intensity of a line and knowing its calculated dipole moment for different conformers can give us an estimation of the energy difference.

6 – The procedure was repeated for several transitions to obtain an averaged result.

7 – To minimize the error, only *b*-type components were chosen, as they are common for both conformers. Using this procedure, the same transitions could be chosen (the degeneracy is the same). Nevertheless, we also made the same estimation including *c*-type transitions for conformer 1 as well as *a*-type transitions for conformer 2 (taking into account the degeneracy), and the results were similar.

Experimental details

A commercial sample of L-Alanyl-L-alanine (m.p. 285 ºC, Sigma Aldrich, ~99%) was used without any further purification. A solid rod was prepared by pressing the compound's fine powder mixed with a small amount of commercial binder and was placed in the ablation nozzle. A picosecond Nd:YAG laser (12 mJ per pulse, 20 ps pulse width) was used as a vaporization tool. Products of the laser ablation were supersonically expanded using the flow of carrier gas (Ne, 13 bar) and characterized by chirped-pulse and molecular beam Fourier transform microwave spectroscopies (LA-CP-FTMW and LA-MB-FTMW).

For the broadband cavity,[7] chirped pulses of 4 µs are generated digitally using an arbitrary waveform generator (AWG) and amplified to about 200 W by pulsed traveling wave tube amplifier (TWT). The resultant pulses are then transmitted and detected by broadband microwave horn antennas in a high-vacuum chamber where they interact with the molecular supersonic expansion. About 120000 free induction decays (4 FID emissions per gas pulse) were averaged and digitized coherently by a 50 GS/s digital oscilloscope. A Kaiser-Bessel window was applied to increase the baseline resolution. The resolution achieved is 50 kHz, and the estimated accuracy of the frequency measurements is better than 10 kHz.

For the narrowband cavity, a Balle-Flygare-type LA-MB-FTMW spectrometer dedicated to maximize its performance from 2 to 8 GHz was used to analyze the hyperfine structure of the rotational lines.[8] Recently, we have implemented a pulse sequence that allows for multiple FID collection.[9] At a flight time of about 1 ms for the jet propagating along the resonator's axis, about 4 FIDs are recorded per gas pulse without compromising the resolution while an S/N improvement of 2 is achieved. The number of FIDs could be increased up to 8 FIDs, with the corresponding increase in the S/N ratio, but at the cost of lowering the resolution. For this work we used a fourfold FID collection. Due to the collinear disposition between the supersonic jet and the microwave resonator axis, all emission signals appeared to split into Doppler doublets. The rest frequency is the arithmetic mean of the doublets. Up to 500 acquisitions were phase-coherently co-added to achieve reasonable signal to noise ratios (S/N).

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