Structure Elucidation

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Direct Spectroscopic Evidence of Hyperconjugation Unveils the Conformational Landscape of Hydrazides**

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Abstract: *The stereochemistry of hydrazides makes them especially interesting as building blocks for molecular design. An exhaustive conformational analysis of three model hydrazides was conducted in a conformer-selective approach by using a combination of high-level quantum chemistry calculations and vibrational spectroscopy in the gas phase and in solution. The NH stretch frequency was found to be highly sensitive to hyperconjugation, thus making it an efficient probe of the conformation of the neighboring nitrogen atom. This property greatly assisted the identification of the isomers observed experimentally in the conformer pool. A rationalization of the hydrazide conformational landscape is proposed, therefore paving the way for a better characterization of secondary structures in larger systems.*

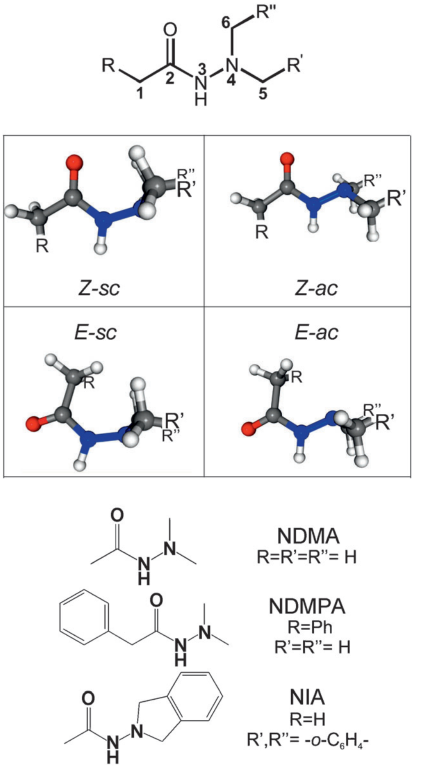

Figure 1. Top: A representative monoacylated hydrazine of the hydrazides investigated herein. Middle: the four intrinsic conformers of the hydrazide group. Bottom: the three hydrazide molecules investigated herein.

Acylated hydrazines (Figure 1) are a widely used family of hydrazides with applications in diverse fields ranging from pharmaceuticals to industrial products and materials.[1,2] Since these building blocks combine the physical and chemical features of hydrazines and peptides, they have generated considerable interest as peptidomimetics,[3] potentially yielding drug candidates with enhanced pharmacological profiles.[4] In monoacylated hydrazines, one nitrogen atom is reminiscent of a planar amide nitrogen atom (N3), whereas the other nitrogen atom (N4) has a free lone pair of electrons, which leads to distinct conformational and physicochemical characteristics. An example of a therapeutic in this compound class is the protease inhibitor atazanavir, which has provided a simplified antiretroviral treatment for human immunodeficiency virus since its introduction a decade ago.[5] Other examples include immunogenic[6] and antimicrobial[7] agents, while in the area of foldamer design, predictions of regular secondary structures for hydrazino peptides[8] have been borne out experimentally.[9–11]

Although the conformational preferences of hydrazides are primordial for their biological activities and an understanding of their origins is of equal importance, appropriate studies have been somewhat limited. Combined theoretical and experimental gas-phase investigations on model compounds are rare.[12] Some solution studies have focused on specific cases, such as the hydrazino turn phenomenon[13–15] or the behavior of cyclic aza-β^3-peptides.[16] It has been suggested that a combination of steric and electronic effects are at play in the conformational preferences of the core N^α-acylated N^β,N^β-disubstituted hydrazide feature.[2,17,18] Given the importance of this central structural feature for the behavior of

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numerous other derivatives, we decided to study it in more detail.

Two stereocenters are involved (Figure 1): the C2–N3 bond, which leads to *E* or *Z* geometries, and the N4 atom, which enables synclinal or anticlinal (*sc* or *ac*) C2-N3-N4-C5 torsional angles (±60° or ±120°, respectively). This leads to a potential energy surface with eight wells, which is reduced to four when R′ = R″, labeled *E-ac*, *E-sc*, *Z-ac*, and *Z-sc*. Hydrazides are often observed as a mixture of two conformers[2,12,17–19] attributed to *E*/*Z* isomerism, although the puzzling appearance of two very different free N3-H stretch frequencies (3424 and 3303 cm^{-1}) in the IR spectra remains unexplained.[19] To better understand this observation and gain deeper insight into the interactions which shape hydrazides, a comprehensive conformational analysis was carried out on three model compounds through a combination of solution and conformer-selective gas-phase IR spectral analysis of the NH stretch region with high-level quantum chemistry calculations of the isolated species.

First, *N′*,*N′*-dimethylacetohydrazide (NDMA) was studied since it is one of the simplest hydrazides which bears only one NH moiety; thus if several transitions appear in the spectral region of the NH stretch, a conformational mixture is implicated. The FTIR spectrum in chloroform indeed shows three transitions (Figure 2): a weak band at 3430 cm^{-1} and two intense bands at 3301 and 3334 cm^{-1}. To conclude that these result from three conformations is not straightforward, however. While the first transition is consistent with an NH group not engaged in a hydrogen bond, the 3300–3350 cm^{-1} range, where the intense doublet lies, is more typical of NH groups engaged in strong hydrogen bonds.[20] It therefore needs to be ascertained whether self-association of NDMA through hydrogen bonding is responsible for this doublet, and if not, why monomers would lead to such unusually red-shifted transitions for nonbonded NH groups.

In this respect, obtaining IR spectra of isolated monomers in the gas phase is of primary interest. To this end, IR/UV double-resonance spectroscopy, a mass-resolved conformer-selective technique,[21] is particularly suitable to record separately the IR spectrum of each conformer of a conformational mixture, provided the system possesses a near-UV chromophore (see the Supporting information). *N′*,*N′*-dimethyl-2-phenylacetohydrazide (NDMPA) and *N*-(isoindolin-2-yl)acetamide (NIA) were designed for such studies. Figure 2 presents both their solution and gas-phase IR spectra. First, their spectra in solution comprise three bands resembling the pattern already described for NDMA. This gives a first indication that the aromatic ring has little interference on the hydrazide function of these systems. Gas-phase experiments reveal that monomers have two conformers, **A** and **B**, with **A** dominating the UV spectrum (see the Supporting information). For both NDMPA and NIA, **A** is characterized by a narrow band in the 3300–3350 cm^{-1} range (3320 and 3330 cm^{-1}, respectively). This observation for isolated molecules in a vacuum proves that the free N3-H group is intrinsically able to reach a transition range normally reserved for strongly hydrogen-bonded NH groups.[20] Transitions of conformer **B** appear in the usual range for a free NH group[20,22] (3456 and 3466 cm^{-1}, respectively).

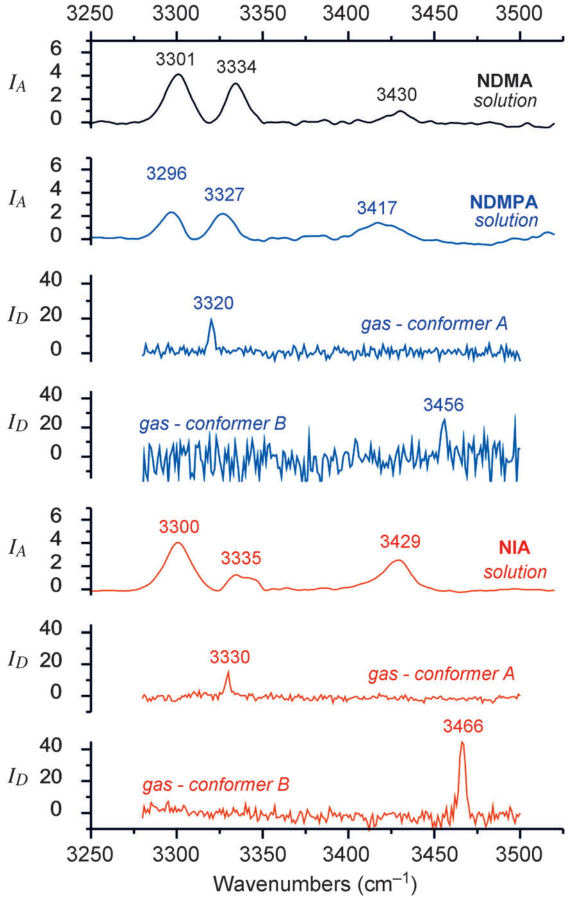

Figure 2. IR spectra of the three hydrazides recorded in solution (FTIR, 10 mM, in $CHCl_3$) and in the gas phase (IR/R2PI-UV). The intensities correspond to absorbance in solution (I_A, $\times 10^{-3}$) or depletion in the gas phase (I_D, %).

To understand these observations, quantum chemistry calculations on the isolated molecules were conducted on the four conformers expected for NDMA, NDMPA, and NIA. In the case of the latter two, the theoretical results can be directly compared to gas-phase data. Energetics and NH stretch frequencies are presented in Table 1. A first point is

Table 1: Energetics and scaled harmonic frequency of the NH stretch.

	NDMA ΔG(300 K)[a]	ν(NH)[b]	NDMPA ΔG(300 K)[b]	ν(NH)[b]	NIA ΔG(300 K)[a]	ν(NH)[b]
E-ac	0	3306	0	3294	0	3303
Z-sc	5	3444	3	3440	3	3436
Z-ac	11	3283	8	3305	10	3285
E-sc	25	3406	29	3404	21	3400

[a] In kJ mol^{-1}. Electronic energies were calculated at the QCISD(T)/TZVPP level on MP2/TZVPP-optimized geometries; thermodynamic corrections at 300 K were calculated at the RI-B97D/TZVPP level on geometries optimized at the same level. [b] In cm^{-1}. A scaling function determined for amide groups was used (see the Supporting Information).

Table 2: NBO analysis.

	NDMA				NDMPA				NIA			
	E-ac	*Z-sc*	*Z-ac*	*E-sc*	*E-ac*	*Z-sc*	*Z-ac*	*E-sc*	*E-ac*	*Z-sc*	*Z-ac*	*E-sc*
$n(\sigma_{N3\text{-}H})$[a]	1.954	1.952	1.948	1.951	1.952	1.951	1.947	1.953	1.955	1.953	1.948	1.952
$n(\sigma^*_{N3\text{-}H})$[a]	0.056	0.038	0.058	0.041	0.057	0.039	0.058	0.042	0.056	0.039	0.058	0.043
$lp_{N4}\rightarrow\sigma^*_{N3\text{-}H}$[b]	51	23	52	29	51	20	49	29	54	24	54	31
$lp_{N4}\rightarrow\sigma^*_{C2\text{-}N3}$[b]	12	53	7	57	11	53	11	58	14	54	8	57

[a] Occupancies were analyzed at the MP2/TZVPP level. [b] Stabilization energies (kJ mol^{-1}) between donor and acceptor orbitals were calculated at the HF/TZVPP level (see the Supporting Information).

that the conformational energetics of all three compounds are very similar, thus validating NDMPA and NIA as good model systems for the characterization of the hydrazide conformational landscape. The results demonstrate that conformers can be classified into two categories according to their NH stretch frequency: conformers *Z-sc* and *E-sc* have frequencies predicted above 3400 cm^{-1}, while *Z-ac* and *E-ac* have frequencies below 3306 cm^{-1}. It appears that the unusual red-shifted signature for the free NH group is associated to the *ac* character of the C2-N3-N4-C5 angle, and not to the *E* or *Z* character of the amide group. Consequently, **A** must be assigned to an *ac* conformer, and **B** to an *sc* conformer. As the lowest energy conformers are expected to be the most populated in the experiment, *E-ac* and *Z-sc* are the most reasonable assignments for **A** and **B**, respectively.

It now has to be explained why conformer **A** has a weaker NH bond than **B**, as evidenced by the considerable red-shift of 136 cm^{-1} in both NDMPA and NIA, and how this is related to the *sc* or *ac* character of the C2-N3-N4-C5 angle. Natural bond orbital (NBO) analyses were carried out and the most significant results are presented in Table 2. For all the conformers of all three systems, the occupancy of the NH bonding orbital ($\sigma_{N3\text{-}H}$) does not vary significantly. However, the occupancy of the NH antibonding orbital ($\sigma^*_{N3\text{-}H}$) dramatically increases by about 40% in *ac* conformers relative to *sc* conformers. It then follows that the red-shift of the predicted NH stretch frequency of the *ac* conformers relative to the *sc* conformers can be ascribed to an increase of the occupancy of the $\sigma^*_{N3\text{-}H}$ orbital. Stabilization energies (Table 2) reveal that this increase in occupancy correlates with an increase in the interaction between $\sigma^*_{N3\text{-}H}$ and the N4 lone pair of electrons (lp_{N4}). This hyperconjugation occurs mostly when the overlap between the $\sigma^*_{N3\text{-}H}$ and lp_{N4} orbitals is optimum, that is, in the *ac* conformers (Figure 3). Another hyperconjugation effect was also revealed between the lp_{N4} and σ^*_{C2N3} orbitals by the same analysis, and occurs mostly in the *sc* conformers. A spectral signature of this latter hyperconjugation should be expected in the CN stretch region, but no effect has been clearly evidenced by harmonic frequency calculations because of strong couplings of the CN stretch with other vibrational modes.

The energetics of hydrazides can be rationalized by comparison with amides. (*Z*)-*N*-methylacetamide is intrinsically more stable than the *E* conformer by 13 kJ mol^{-1} (see the Supporting Information). This energetic order is preserved in *sc* hydrazides (*E-sc* and *Z-sc*), with an energy gap even raised to more than 20 kJ mol^{-1} because of steric clashes in *E-sc* between C5-H$_2$ and C6-H$_2$ on one side, and C1-H$_2$ on the other side.[17] Surprisingly, this order is totally reversed for the *ac* pair, with *Z-ac* being less stable than *E-ac* by about 10 kJ mol^{-1}. The analysis of conformer geometry reveals that all NDMA conformers except *Z-ac* have a plane of symmetry (C2N3N4 plane). The loss of symmetry in *Z-ac* is due to the rotation around the NN bond caused by the electronic repulsion between the lone pair of electrons on the oxygen atom (lp_O) and lp_{N4} (Figure 3).[17,18] Therefore, *Z-ac* is shifted about 20 kJ mol^{-1} higher relative to *E-ac* than is expected for a *E*/*Z* pair. Without this repulsion, *Z-ac* would be expected to be 13 kJ mol^{-1} lower in energy than *E-ac* (*E*/*Z* amide energy gap), and all the *ac* conformers would be far more stable than their *sc* counterparts by at least 15 kJ mol^{-1} (Table 2). The differential hyperconjugation effect between $lp_{N4}\rightarrow\sigma^*_{N3\text{-}H}$ stabilizing the *ac* conformers, and $lp_{N4}\rightarrow\sigma^*_{C2\text{-}N3}$ stabilizing the *sc* conformers must contribute to this intrinsic energy difference between the *ac* and *sc* conformers, although it is

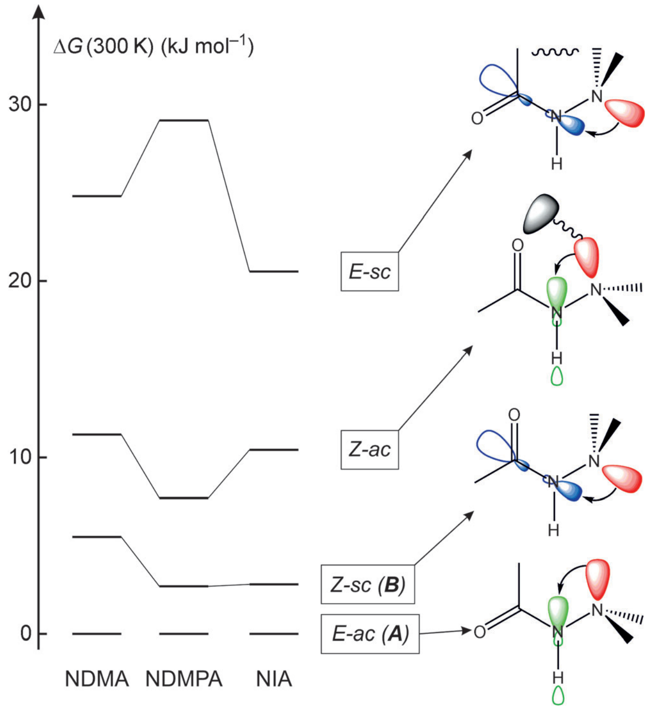

Figure 3. Energetics of NDMA, NDMPA, and NIA conformers. The conformational landscape of hydrazides is mainly governed by hyperconjugation effects between σ^*_{NH} (green) and lp_{N4} (red), lp_{N4} and σ^*_{C2N3} (blue), the electronic repulsion between lp_O (gray) and lp_{N4} in *Z-ac*, and a steric clash in *E-sc* (see text for details).

not possible to quantify it from the level of calculation employed. Finally, while *E-sc* and *Z-ac* are too energetically penalized to be observed in the gas phase, solvent effects may bring the energy of *Z-ac* low enough to be observed in solution, as revealed by FTIR spectra.

In conclusion, this study reveals that hyperconjugation between the $\sigma^*_{\mathrm{N3\text{-}H}}$ and $\mathrm{lp}_{\mathrm{N4}}$ orbitals has a huge impact on the NH stretch frequency in model hydrazides. Large red-shifts (ca. 130 cm^{-1}) relative to the habitual free NH stretch range have been observed and are ascribed to *ac* conformers (hyperconjugation-induced shifts of less than 40 cm^{-1} have been previously observed for OH and CH stretches in 1,1,1,3,3,3-hexafluoro-2-propanol in CCl_4[23]). This phenomenon may be a useful spectroscopic tool for conformational assignment purposes in more complex hydrazides. It may be also pointed out that the vibrational signature of hyperconjugation is rarely used for conformational analysis,[23,24] despite the ubiquity of hyperconjugation[25,26] and the high added-value of such a characterization. Besides hydrazides,[19] large hyperconjugation-induced red-shifts can be anticipated in biologically relevant molecules, as suspected from free NH stretch calculations on a tetrahydroisoquinoline derivative.[27] The reason why the vibrational signature of hyperconjugation is too often neglected might come from its general occurrence in spectral regions that become easily crowded in large species. The present demonstration of the value of direct spectroscopic evidence of hyperconjugation may be expected to foster both experimental and theoretical developments leading to a better characterization of such effects through their vibrational signatures.

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