J. Org. Chem. 1993, 58, 6397–6405

Theoretical Study of *N*-Methylacetamide in Vacuum and Aqueous Solution: Implications for the Peptide Bond Isomerization

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Received May 11, 1993[●]

A theoretical study on the changes in energy and charge distribution upon rotation of the amide bond of *N*-methylacetamide is presented. Both gas-phase and aqueous environments have been simulated in semiempirical (AM1) and *ab initio* (STO-3G, 6-31G and 6-31G*) calculations with inclusion of electron correlation effects at the Moller–Plesset level. Relevant changes in the charge distribution are found during rotation of the peptide bond. The inclusion of water largely affects not only the energetics and the pathway of the amide twisting but also the charge distribution of the different conformers. Results are in good agreement with available experimental data and with results derived from high-level theoretical calculations. The chemical and biochemical importance of the results is discussed.

Introduction

Resonance is one of the most relevant concepts in chemistry because it explains a large number of molecular properties, like the planarity of conjugated bonds, which exhibit a mixed character between a single and a double bond related to the sp^2 hybridization of the bonded atoms. One of the most important conjugated bonds is the amide bond, which is responsible for the sequential linkage between the amino acids. Understanding of the chemical nature of the peptide bond is essential to explain the structure and dynamics of proteins and other polymers.

The amide bond adopts a *trans* conformation in most proteins, although the *cis* conformation is also possible when there is proline on the nitrogen side. Early NMR experiments showed that the *cis* ↔ *trans* interconversion is a highly unfavored reaction with energy barriers in the range of 15–20 kcal/mol.[1] This suggested that the *trans* ↔ *cis* interconversion is highly improbable and has little biological significance. Nevertheless, this picture changed after the statement of the importance of the *trans* ↔ *cis* prolyl isomerization in the folding of different proteins[2] and the determination that the cytosolic enzymes involved in such process (*peptidyl-prolyl isomerases*) are abundant in both eucaryotes and procaryotes. These enzymes have also been related to immunological[3–5] and visual transduction phenomena.[6]

From a chemical point of view, the *cis* ↔ *trans* interconversion involves the disruption of the amide resonance, and the rotational barrier can be understood by the loss of the π-conjugated bond. According to the classical resonance model,[7] such disruption would lead to a large charge transfer, in such a way that the nitrogen is enriched in electrons, whereas the negative charge in the oxygen decreases. In addition, large geometric changes occur, since the change of hybridization from sp^2 to sp^3 in the nitrogen elongates the C–N bond, whereas the C–O bond is shortened.

This model explains several properties of amides, but it has been criticized[8] since results derived from *ab initio* calculations pointed out some inconsistencies. Among them, (i) only small changes in the C–O distance occur when the bond rotates, (ii) the electron population at the nitrogen, as computed from Bader's analysis, decreases in twisted structures, (iii) the electron population at the carbonyl oxygen does not change significantly upon rotation, and (iv) force constants for the C–O bond do not change during the amide rotation. Accordingly, an alternative resonance model has been proposed in which

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[●] Abstract published in *Advance ACS Abstracts*, September 15, 1993.

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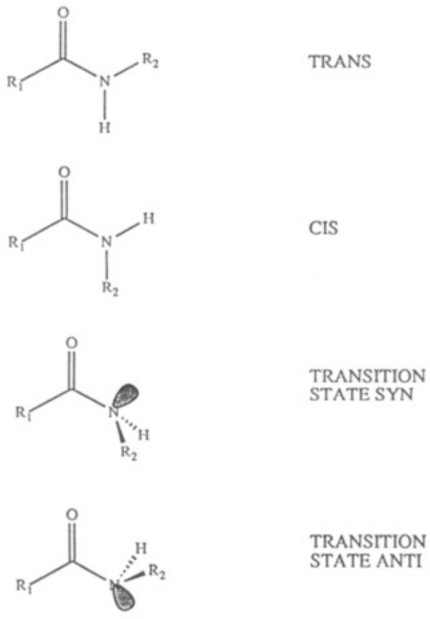

Figure 1. Schematic representation of the four stationary points for the rotation of the amide bond.

the rotational barrier is mainly due to the shift of electron density from the nitrogen to the carbonyl carbon.[8]

Whatever the mechanism involved in the peptide bond rotation, large changes in the electrostatic distribution should occur. Inspection of the four stationary conformers around the amide bond (Figure 1) suggests that the dipole moment strongly varies upon rotation. Moreover, since the two models assume a charge transfer along the C–N bond, the atomic monopoles would change, and this would alter the electrostatic properties. The shift in the charge distribution stresses the solvent effect on the energetics of the rotation, since polar solvents like water stabilize polar structures. Therefore, major changes in the thermodynamics and kinetics of the amide rotation are expected upon changes in environment. This may be of biological relevance, since it has been suggested that the enzymatic acceleration of the peptidyl prolyl isomerization largely stems from the vacuum-type environment in which the reaction occurs.[9]

The solvent effect was largely studied with NMR techniques from the late 60's to early 80's. These studies, mainly performed on N,N-disubstituted amides, showed an increase in the isomerization barrier in water of 3–5 kcal/mol, while the *cis–trans* ratio remained little affected. Recently, the *cis–trans* equilibrium has been explored theoretically in the gas phase[8,10] and in solution.[10a,d,e,h–i] The most recent studies are the *ab initio* and MonteCarlo calculations on the *N*-methylacetamide (NMA) by Gao and Jorgensen[10h] and the *ab initio* and Molecular Dynamics studies on the same molecule by Kollman's group.[10i] In both cases free energy perturbation techniques[11] were used to compute the difference between the ΔG of solvation of *cis* and *trans* isomers, which was added to the gas-phase ΔG between those conformers for determining the ΔG of isomerization in water. Finally, MonteCarlo PMF techniques have been applied to analyze the solvent effect on the kinetics of dimethylacetamide (DMA) isomerization.[10k]

In this paper a theoretical study of the change in energy and charge distribution of NMA is presented. Both semiempirical (AM1; ref 12) and *ab initio* (up to 6-31G*; ref 13) methods with inclusion of Moller–Plesset electron correlation effects[14] are used. Each conformation of the NMA (*cis*, TSsyn, TSanti, and *trans*) is studied in vacuum and aqueous solution, the latter being modeled using Tomasi's SCRF algorithm[15] implemented in both *ab initio* and semiempirical frameworks.[15c] From these calculations we expect to gain insight into different aspects of the peptide bond rotation: (i) the energetics and (ii) the change in charge distribution during the C–N rotation, (iii) the effect of water on the thermodynamics and kinetics of the isomerization reaction, (iv) the effect of water on the charge distribution of the different conformations, and finally (v) the ability of low level *ab initio* methods, or even of the semiempirical AM1, to reflect results derived from accurate *ab initio* calculations.

Methods

Molecular Structure and Energy Calculations. NMA was considered in four conformations: the two minima, *cis* and *trans*, and the two transition states, TSsyn and TSanti (Figure 1). Geometry optimizations were performed at the AM1[12] and *ab initio* STO-3G,[16] 6-31G,[17] and 6-31G*[13] levels. Force constant analyses were carried out to verify the minimum or transition state nature of the conformations. In some cases, particularly for the STO-3G basis, the minimum appeared as a nonplanar structure due to an overestimation of two-electron repulsion interactions. In these cases, the minimum (C1) and also the most stable planar structure determined by imposing the C_s symmetry (CS) were computed. Moller–Plesset perturbation treatment[14] was used to compute electron correlation corrections to the energy from the structures optimized at the 6-31G* level because they are expected to give accurate geometries.[8a,10h] MP2 corrections were computed from the 6-31G* basis. The small correction up to the MP4 level was computed at the 6-31G level and added to the MP2/6-31G* value.[18] The final value is denoted as MP4$^{\neq}$/6-31G*.

The thermodynamics in vacuum was computed by correcting the differences in electronic energy to enthalpies at 298 K[19] using eq 1,[20] where $\Delta E^0_{\rm ele}$ is the electronic energy difference, $\Delta E^0_{\rm vib}$ and

$$\Delta H^{298} = \Delta E^0_{\rm ele} + \Delta E^0_{\rm vib} + \Delta E^0_{\rm rot} + \Delta\Delta E^{298}_{\rm vib} + \Delta\Delta E^{298}_{\rm rot} + (\Delta PV) \quad (1)$$

$\Delta E^0_{\rm rot}$ are the vibrational and rotational corrections at 0 K, and $\Delta\Delta E_{\rm vib}{}^{298}$ and $\Delta\Delta E_{\rm rot}{}^{298}$ are the thermal corrections. These terms

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were computed from AM1 calculations following the standard MOPAC thermodynamic analysis,[21] the expansion term being negligible. In our experience, corrections at this level provide similar results to those derived from high-level *ab initio* calculations, thus avoiding the use of scaling processes, which may be debatable when different *ab initio* wavefunctions are considered. The close agreement between our AM1 values and *ab initio* results reported by other authors supports the suitability of this approach (see Results and Discussion).

The entropic correction to the enthalpy was also computed from AM1 frequency analysis following the standard formulas (the imaginary frequency at the transition state was removed from the frequency analysis), and excellent agreement with *ab initio* corrections was also found (see Results and Discussion). Finally, the free-energy difference between conformers was computed according to eq 2.

$$\Delta G^{298} = \Delta H^{298} - T\Delta S^{298} \qquad (2)$$

Molecular Electrostatic Potential and Charge Calculations. Electrostatic properties were analyzed not only from the dipole moments but also from the molecular electrostatic potential (MEP; ref 22) and the MEP-derived charges. The procedure developed in our laboratory was used to compute the MEP from AM1 wavefunctions.[23] Previous studies validated the SCF monodeterminantal wavefunction[24] because the effect of electron correlation on the MEP and MEP-derived properties is very small.

Electrostatic (potential-derived) charges were determined following Momany's procedure,[25] where the quantum mechanical MEP is fitted to a set of monopoles centered at the nuclei. The selection of points where the quantum mechanical and coulombic MEPs are fitted plays a key role.[26] The use of square lattices leads to unreliable results, since charges depend on rotations about the external axis.[26c] Thus, we followed the strategy suggested by Singh and Kollman,[26b] where the points are selected in Connolly layers[27] outside the van der Waals radii of the atoms. This algorithm gives reliable charges when a density of points up to 1 point/Å^2 is used.[26a] Nonetheless, in order to detect small variations in charges due to conformational changes or to the environment, a preliminary study in which charges were determined at increasing densities of points was carried out. For every density, the variation of charges with respect to lower and higher density calculations was evaluated. This procedure was repeated until no change was found. Convergence appeared for a density of 10 points/Å^2 using two layers at 1.4 and 1.8 times the van der Waals radii of the atoms. This value was used in all the remaining fittings. The use of two layers instead of the four layers recommended by Singh and Kollman[26b] does not introduce errors in the charges but reduces the computational cost by a factor of 2.[26a] No dipole restriction was imposed in the fitting, which allows the SCF and electrostatic dipoles to be compared so as to analyze the quality of the electrostatic charges.

Solvation Calculations. The effect of water on the energy and charge distribution of the conformers was examined following the Miertus–Scrocco–Tomasi strategy,[15] which gives accurate representations of the solvent effect on the molecular charge distribution.[15d] This SCRF strategy computes the total free energy of solvation as the addition of three contributions: cavitation, van der Waals, and polarization (eq 3):

$$\Delta G_{\text{solv}} = \Delta G_{\text{cav}} + \Delta G_{\text{vw}} + \Delta G_{\text{pol}} \qquad (3)$$

The cavitation term can be computed from classical particle theory according to different algorithms, like those of Pierotti[28] or Sinanoglu.[29] In this study, Pierotti's method was used at 298 K, and the standard parameters of water were considered.[28] Some calculations were also performed following Sinanoglu's method,[29] and similar results were obtained. The van der Waals contribution was evaluated from the linear correlation with the solute surface reported by Tomasi and co-workers.[30]

The polarization term was determined in an SCRF procedure, where the effect of water is represented by a set of virtual charges $\{Q_i\}$ located on the molecular cavity surface (eq 4, where H^0 is the

$$H = H^0 + \sum_i \frac{Q_i}{R_i} \qquad (4)$$

solute nonperturbed Hamiltonian). These charges are determined from the gradient of the MEP at the cavity surface by solving the Laplace equation. The introduction of the perturbation operator into the solute Hamiltonian requires an iterative procedure to solve the Schrödinger equation. In contrast to other SCRF methods, where truncated multipole expansions are used,[31] this model gives a rigorous formulation of the perturbation operator, where all the multipole interaction terms are explicitly considered. Furthermore, the solute cavity is not represented by a single sphere or ellipsoid,[31,32] but it is built according to the molecular shape following Ahuir *et al.*,[33] which makes it suitable for the study of nonspherical molecules like NMA. These features enable the model to give a precise representation of the solute–solvent interaction. Nevertheless, the large computational cost may be a serious shortcoming.

The polarization term was calculated from the SCF wavefunction. The standard Tomasi' strategy[15a,b] implemented in the Monstergauss program was used in *ab initio* calculations. A semiempirical-adapted version was used for AM1 calculations.[15c] This version is based on previous studies on semiempirical MEPs,[23] and it provides results close to the values derived at the *ab initio* level. The selection of the cavity is crucial in any continuum model, since results, especially the ΔG of solvation, largely depend on the cavity size. According to previous studies,[15] the cavity was located at 1.2 times the van der Waals radii of the different atoms (standard parameters H, 1.2 Å, C, 1.5 Å, and O 1.4 Å were considered).

The free energy of reaction in water was evaluated by the classical thermodynamical scheme:

$$\Delta G^{\text{reac}}_{\text{aq}} = \Delta G^{\text{reac}}_{\text{vac}} + \Delta\Delta G_{\text{solv}} \qquad (5)$$

Semiempirical calculations were performed with a modified version[20,34] of the MOPAC program, which includes an additional code to compute MEPs and solvent effects. *Ab initio* calculations were performed with HONDO-77[35] and MONSTERGAUSS[36] computer programs. All the calculations were performed on the IBM-3090 at the Centre de Supercomputació de Catalunya (CESCA).

Results and Discussion

Vacuum Energy Calculations. 6-31G*-optimized structures are shown in Figure 2. The STO-3G optimizations provided "out of the plane" minimum structures, but AM1 and 6-31G gave geometries close to the 6-31G* ones. Figure 2 shows the change from sp^2 to sp^3 hybrid-

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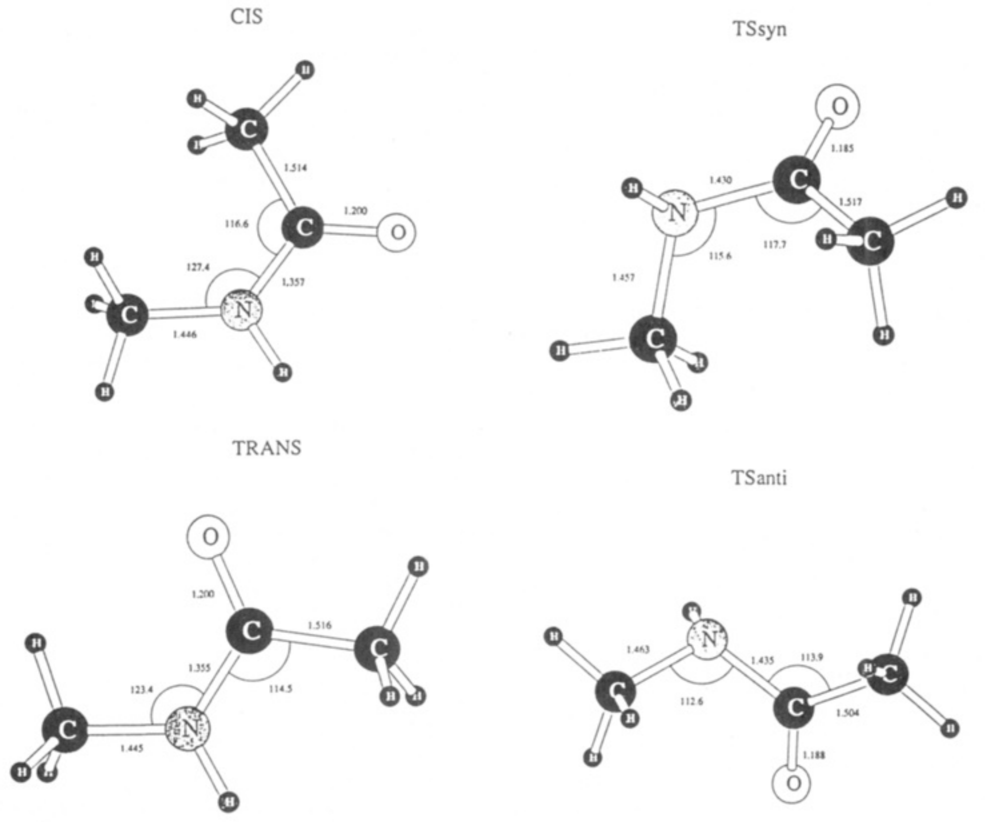

Figure 2. 6-31G*-optimized geometries of the four stationary structures. Bond lengths (Å) and angles (deg) are shown.

Table I. Energy Differences (in kcal/mol) in Vacuum Between the Four Conformations of NMA Computed from AM1, STO-3G, 6-31G, 6-31G*, MP2/6-31G* and MP4$^{\#}$/6-31G*^{a}

		TSsyn	TSanti	*trans*
AM1	*cis*	−12.14	−8.70	0.17
	TSsyn		3.44	12.31
	TSanti			8.87
STO-3G	*cis*	−8.95	−6.62	1.36, −0.38
		−6.84	−4.50	3.48, 1.74
	TSsyn		2.34	10.30, 8.57
	TSanti			7.98, 6.24
6-31G	*cis*	−23.60	−17.81	2.36, 2.35
	TSsyn		5.79	25.92, 25.91
	TSanti			20.21, 20.20
6-31G*	*cis*	−18.32	−13.76	2.30
	TSsyn		4.56	20.62
	TSanti			16.06
MP2/6-31G*	*cis*	−18.99	−14.61	2.22
	TSsyn		4.37	21.21
	TSanti			16.83
MP4#/6-31G*	*cis*	−18.67	−14.42	2.23
	TSsyn		4.25	20.90
	TSanti			16.65

[a] Values are obtained as energy of conformer (row) − energy of conformer (column). When two minima exist, both energy values are reported (top: C1, bottom: CS). When both (row and column) conformations present two minima, four values are shown (left top: C1–C1; left bottom: CS–C1; right top: C1–CS; right bottom: CS–CS).

Table II. Differential Thermal (Roman Text) and Entropic (Italic Text) Corrections (in kcal/mol) at 298 K to the Energies. Values Are Obtained as Correction of Conformer (Row) − Correction of Conformer (Column)

	TSsyn	TSanti	*trans*
	0.54	0.56	−0.09
cis	*−1.24*	*−1.29*	*0.40*
		0.02	−0.63
TSsyn		*−0.05*	*1.64*
			−0.65
TSanti			*1.68*

ization at the transition states, as noted by the C–N–C–C dihedral angles and the C–N–C bond angles. The C–N bond length elongates around 0.08 Å when the conjugation is broken, and the C–O distance has a small variation around 0.013 Å.

Energy differences are shown in Table I. In all cases the *trans* is the most stable conformer, but AM1 seems to underestimate the *cis*–*trans* energy difference (0.17 kcal/mol as compared to 2.23 kcal/mol at the MP4$^{\#}$/6-31G* level). A smaller underestimation is also found in C1 (1.36 kcal/mol) and CS (1.74 kcal/mol) STO-3G structures. The TSanti is the most favored route for the *cis* ↔ *trans* interconversion. The energy difference between the two transition states is underestimated in STO-3G calculations, but AM1 and 6-31G results qualitatively agree with those derived at the MP4$^{\#}$/6-31G* level, for which the energy barrier for the *cis* to *trans* isomerization *via* TSanti is 14.42 kcal/mol, while the *trans* to *cis* is 16.65 kcal/mol. AM1, and especially the STO-3G basis, underestimate such a barrier, which is overestimated in 6-31G calculations. It is worth noting the small effect of electron correlation, which lies in the range 3–6% of the total energy difference.

Enthalpic corrections and the $-T\Delta S$ entropic contribution are shown in Table II. Corections to the enthalpy are small except when minima and transition states are compared, since values around 0.6 kcal/mol are found. Such corrections decrease the isomerization enthalpy barriers. The entropic correction is generally significant, even when minimum structures are compared, for which a variation of 0.40 kcal/mol is obtained. Moreover, the entropic contribution notably increases the rotational barrier (around 1.6–1.7 kcal/mol at 298 K). The experimental determination of the entropic term is difficult, since errors are usually larger than the measured value.[1] Nevertheless, an entropic effect of 0.42 kcal/mol at 298 K from *ab initio* 3-21G frequency analysis has been

Table III. Free Energy Differences (in kcal/mol) at 298 K in Vacuum between the Four Conformations of NMA Computed from AM1, STO-3G, 6-31G, 6-31G*, MP2/6-31G* and MP4#/6-31G*. Values Are Obtained as ΔG of Conformer (Row) – ΔG of Conformer (Column) (See Footnote *a* in Table I).

		TSsyn	TSanti	*trans*
AM1	*cis*	−12.84	−9.44	0.48
	TSsyn		3.41	13.31
	TSanti			9.90
STO-3G	*cis*	−9.65	−7.36	1.67, −0.07
		−7.53	−5.22	3.79, 2.05
	TSsyn		2.31	11.33, 9.58
	TSanti			9.02, 7.29
6-31G	*cis*	−24.30	−18.54	2.67, 2.66
	TSsyn		5.76	26.91, 26.90
	TSanti			21.2, 21.3
6-31G*	*cis*	−19.02	−14.50	2.61
	TSsyn		4.53	21.62
	TSanti			17.09
MP2/6-31G*	*cis*	−19.69	−15.35	2.53
	TSsyn		4.34	22.21
	TSanti			17.86
MP4#/6-31G*	*cis*	−19.37	−15.16	2.54
	TSsyn		4.22	21.91
	TSanti			17.68

reported,[10h] which agrees with our semiempirical value (0.40 kcal/mol). Indeed, studies of the entropic contribution to the rotational barrier in prolyl-peptides suggest that our theoretical value is quite accurate.[37] Furthermore, the entropic term in DMA from 6-31G* frequencies[10k] was estimated to be around 1 kcal/mol at 298 K. This agreement is also found in the enthalpic correction of −0.08 kcal/mol at the 3-21G level reported by Gao and Jorgensen[10h] with regard to our estimate of −0.09 kcal/mol.

The ΔG^{298} for the isomerization in vacuum are given in Table III. Inclusion of enthalpic and entropic corrections introduces numerical changes in the results, but the behavior of the different methods is the same. Results show the enthalpic nature of the isomerization in agreement with NMR experiments.[1] The free energy variation between *cis* and *trans* at the highest computational level is 2.54 kcal/mol. Such a difference closely agrees with the experimental value of 2.5 ± 0.4 kcal/mol and with results derived from calculations at the MP2/6-31G* and 6-31G** levels,[10h,38] it being closer to experimental data than previous theoretical values.[10] The TSanti is the most stable transition state by 4.22 kcal/mol (ΔE^0: 4.25 kcal/mol). A similar value was reported for DMA,[10k] for which the difference of ΔG at 298 K between the two transition states was 4.1 kcal/mol. The preference for the TSanti in vacuum may be explained by the larger charge separation in the TSsyn.[8a]

The free energy barriers for the *cis*-to-*trans* interconversion are 15.16 (TSsyn) and 19.37 (TSanti) kcal/mol, while barriers for the *trans*-to-*cis* isomerism are 17.68 (TSanti) and 21.91 (TSsyn) kcal/mol. NMR studies in 1,2-dichloroethane[1h] provided values of 18.0 ± 0.3 (*syn*) and 20.8 ± 0.3 (*anti*) kcal/mol. These values may be slightly overestimated due to aggregation effects, which would improve the agreement of our results with experimental data. Furthermore, the gas-phase free energy barrier is 15.6 kcal/mol for dimethylacetamide,[1k] 18–19 kcal/mol for formamide, and 15–19 kcal/mol for other N-disubstituted amides.[1d–n,37] MP3/6-31G* calculations for formamide[8a] reported rotational barriers of 15.3 (TSanti) and 17.6 (TSsyn) kcal/mol. MP4/6-31G* calculations on DMA[10k] showed a free energy of isomerization in the range of 14.6–18.7 kcal/mol, also in good agreement with our estimates.

The preceding discussion states that calculations at the MP4#/6-31G* level correctly represent the gas phase rotation around the peptide bond. Comparison with less sophisticated (less expensive) calculations indicates that in general 6-31G* is the minimum level of theory needed to obtain reasonable values. Thus, the 6-31G overestimates the torsional barrier by 18–20%. AM1 is not able to represent the *cis–trans* preference and underestimates the barriers by 35–45%, although suprisingly good estimates of the relative TSanti–TSsyn stability are obtained. Finally, the STO-3G cannot reflect even the planarity of the peptide bond and largely underestimates the torsional barriers.

Energy Calculations in Water. The representation of solvent effects in continuum models depends on the cavity size. No clear criteria exist to define the cavity,[39] which must be carefully selected.[40] Our approach was to use a molecule-shaped cavity, for which the highest level of calculation available (6-31G*) provided good estimates of the experimental ΔG of solvation. Such a cavity was built by scaling the van der Waals radii of the atoms. Since the ΔG of solvation of NMA is not available, acetamide was chosen as a test due to its similarity to NMA and because experimental estimates of its solvation free energy are known.[41]

SCRF results point out that the increase in the cavity decreases the ΔG of solvation, mainly due to the reduction of the polarization term (plots are available from the authors upon request). The scaling of the van der Waals radii by a factor of 1.2 gives the best representation, since the total free energy of solvation is predicted with an error of a few tenths of kcal/mol. This scaling factor generates a cavity with physical meaning, since it can be related to the first solvation shell. In this respect, MonteCarlo simulations[42] have shown that water molecules of the first hydration shell are at average distances equal to the van der Waals atomic radii of the closest atom plus the van der Waals radii of water. Comparison of Molecular Dynamics and Tomasi's (6-31G*) results has shown that the first solvation shell can be effectively simulated from the scaling of the van der Waals radii of the solute atoms by a factor of 1.2.[15e] Therefore, such a scaling factor was used in all the calculations.

The ΔG of solvation (in absolute values) at the 6-31G* level are displayed in Table IV, where the hidrophilicity order TSsyn > *cis* > *trans* ≫ TSanti is stated. Results indicate the NMA is less solvated than acetamide by 2.7 kcal/mol due to the replacement of an acidic hydrogen by a methyl group. This effect can be roughly estimated by

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Table IV. Contributions (in kcal/mol) to the ΔG of Solvation of NMA Computed at the 6-31G* Level. A Cavity Size Equal to 1.2 Times the van der Waals Atomic Radii Was Used. The Temperature Was Taken Equal to 298 K.

	ΔG_{sol}, pol	ΔG_{sol}, cav	ΔG_{sol}, vw	ΔG_{sol}, cav + vw	ΔG_{sol}, total
cis	−10.76	12.70	−9.45	3.25	−7.51
TSsyn	−10.81	12.86	−9.63	3.23	−7.58
TSanti	−7.88	13.16	−9.84	3.32	−4.56
trans	−10.30	12.97	−9.68	3.29	−7.01

Table V. Differential ΔG of Solvation (in kcal/mol) at 298 K between the Different Conformations of NMA Computed from SCRF Calculations at the AM1, STO-3G, 6-31G, and 6-31G*. Values Are Obtained as ΔG of Solvation of Conformer (Row) − ΔG of Solvation of Conformer (Column) (See Footnote *a* in Table I)

		TSsyn	TSanti	*trans*
AM1	*cis*	−0.28	−2.19	−0.62
	TSsyn		−1.91	−0.34
	TSanti			1.56
STO-3G	*cis*	1.18	−0.53	0.03, −0.26
		1.19	−0.52	0.04, −0.25
	TSsyn		−1.71	−1.15, 1.44
	TSanti			0.56, 0.27
6-31G	*cis*	−0.13	−3.38	−0.89, −0.67
	TSsyn		−3.24	−0.76, −0.54
	TSanti			2.48, 2.70
6-31G*	*cis*	0.07	−2.95	−0.50
	TSsyn		−3.02	−0.57
	TSanti			2.45

Table VI. Free Energy Differences (in kcal/mol) at 298 K in Water between the Four Conformations of NMA Computed from AM1, STO-3G, 6-31G, 6-31G*, MP2/6-31G*, and MP4#/6-31G*. Values Are Obtained as ΔG of Conformer (Row) − ΔG of Conformer (Column) See Footnote *a* in Table I)

		TSsyn	TSanti	*trans*
AM1	*cis*	−13.12	−11.63	−0.14
	TSsyn		1.50	12.97
	TSanti			11.46
STO-3G	*cis*	−8.47	−7.89	1.70, −0.33
		−6.34	−5.74	3.83, 1.80
	TSsyn		0.60	10.17, 8.14
	TSanti			9.58, 7.56
6-31G	*cis*	−24.43	−21.92	1.78, 1.99
	TSsyn		2.52	26.14, 26.36
	TSanti			23.71, 24.02
6-31G*	*cis*	−18.95	−17.45	2.11
	TSsyn		1.51	21.05
	TSanti			19.54
MP2/6-31G*	*cis*	−19.62	−18.30	2.03
	TSsyn		1.32	21.64
	TSanti			20.31
MP4#/6-31G*	*cis*	−19.32	−18.11	2.04
	TSsyn		1.20	21.34
	TSanti			20.13

comparing the ΔG of solvation of unionized acids and esters, for which a $\Delta\Delta G$ of solvation around 3 kcal/mol was reported.[41] Therefore, our ΔG of solvation of NMA seems reasonable, probably better than previous SCRF estimates.[43,44] To our knowledge, there is only one MonteCarlo study on the calculation of the total ΔG of solvation of NMA: that reported by Honig and coworkers,[45] who determined estimates of −10.1 (*cis*) and −7.4 (*trans*) kcal/mol from perturbation theory. These results, however, do not agree with experimental values of the relative ΔG of solvation or with the relative free energy of solvation ($\Delta\Delta G$) determined from MonteCarlo and Molecular Dynamics simulations.[10h,i] An inaccurate sampling or the common set of charges used for both conformers may explain this discrepancy.

The different basis sets, except the STO-3G, and the AM1 provide similar results (Table V). This agreement is encouraging, since it demonstrates that the selection of a suitable cavity enables to obtain reasonable estimates of the $\Delta\Delta G$ of solvation between conformers, even from methods unable to represent the electrostatic properties accurately.[23,46] The *cis* conformer is stabilized relative to the *trans* by 0.5 kcal/mol (6-31G*), 0.67 (6-31G), and 0.62 kcal/mol (AM1). This small $\Delta\Delta G$ difference, which may be due to the low dipole difference between *cis* and *trans*, is quite reasonable according to experimental data by Drakenberg and Forsen,[1h] who reported a stabilization of the *cis* of 0.3 kcal/mol when the solvent changes from 1,2-dichloroethane to water. Recently, Wolfenden and coworkers[38] reported an effect on the *cis–trans* equilibrium in the range of 0.1 kcal/mol. MonteCarlo studies,[10h] where different charges were used for *cis* and *trans* conformations, provided $\Delta\Delta G$ of solvation for the *cis–trans* equilibrium close to 0. A similar value was also found by Kollman and co-workers, who used 6-31G* electrostatic charges fitted at both *cis* and *trans* conformations and a modified set of van der Waals parameters.[10i]

The ΔG of reaction (see eq 5) are shown in Table VI (values at the Moller–Plesset level were derived by adding the 6-31G* $\Delta\Delta G$ of solvation to the corresponding ΔG in vacuum). Table VI shows the preference of the *trans* over the *cis* conformer by 2.04 kcal/mol at the highest computational level. Similar results are found for the MP2/6-31G* (2.03), 6-31G* (2.11), 6-31G (1.99), and STO-3G (1.80). As expected from results in gas phase, AM1 does not reflect the *cis–trans* preference satisfactorily.

Results in Table V show that the TSanti is destabilized with regard to the *trans* minimum by 2.45 kcal/mol (6-31G*), 2.70 (6-31G), and 1.56 kcal/mol (AM1). The stability of the TSsyn with respect to the *trans* is increased by 0.57 (6-31G*), 0.54 (6-31G), and 0.34 kcal/mol (AM1). These results show the important role of water on the *cis–trans* isomerism, since the TSsyn is stabilized around 3 kcal/mol with regard to the TSanti. The TSanti is the preferred route for the *cis* ↔ *trans* interconversion (Table VI), but the preference is only of 1.2 kcal/mol at the highest level (MP2/6-31G*, 1.32; 6-31G*, 1.51; 6-31G, 2.52; STO-3G, 0.60; AM1, 1.50 kcal/mol). Therefore, the *trans* to *cis* conversion has a free energy barrier in water of 20.13 kcal/mol at the MP4#/6-31G* (2.45 kcal/mol larger than in vacuum), whereas the *cis* to *trans* barrier is 18.11 kcal/mol (around 3 kcal/mol larger than in vacuum).

Experimental measurements suggest ΔG of activation of 18.8 (*cis*-to-*trans*) and 21.3 (*trans*-to-*cis*) kcal/mol, respectively. These values agree with our results at the highest computational level (*cis*-to-*trans*: 18.11–19.32 kcal/mol; *trans*-to-*cis*: 20.13–21.34 kcal/mol). In addition, NMR studies of N,N-disubstituted amides and prolylpeptides point out that the ΔG of activation for the isomerization in water is around 19 kcal/mol, the barrier being increased with regard to apolar solvents around 2.5 kcal/mol.[1,41] High-level *ab initio* estimates for DMA[10k] indicate that the stability of the TSsyn with respect to the TSanti increases around 4 kcal/mol in water. Furthermore,

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Table VII. Electrostatic Charges (in Units of Electron), SCF, and Electrostatic Dipoles (D) for the Different Conformations of NMA in Vacuum and Aqueous Solution Computed from the AM1, STO-3G, 6-31G, and 6-31G* Wave Functions

		AM1		STO-3G		6-31G		6-31G*	
		vacuum	water	vacuum	water	vacuum	water	vacuum	water
cis minima	N	−0.399	−0.387	−0.718	−0.731	−0.662	−0.667	−0.562	−0.572
	C(O)	0.360	0.379	0.807	0.830	0.915	0.988	0.787	0.848
	O	−0.349	−0.456	−0.450	−0.492	−0.743	−0.889	−0.656	−0.782
	HN	0.293	0.302	0.310	0.316	0.368	0.383	0.330	0.345
	C(C)	0.014	0.048	−0.124	−0.114	−0.051	−0.022	−0.043	−0.016
	C(N)	0.081	0.113	0.176	0.192	0.173	0.207	0.144	0.177
	μSCF	3.83	5.11	2.35	2.81	4.82	6.34	4.20	5.54
	μELE	3.24	4.66	2.38	2.83	4.88	6.43	4.29	5.65
cis CS minima	N			−0.445	−0.443				
	C(O)			0.647	0.669				
	O			−0.446	−0.498				
	HN			0.248	0.252				
	C(C)			−0.081	−0.069				
	C(N)			0.077	0.089				
	μSCF			2.66	3.25				
	μELE			2.71	3.29				
TSsyn	N	−0.707	−0.744	−0.736	−0.760	−1.013	−1.102	−0.889	−0.986
	C(O)	0.480	0.523	0.806	0.834	1.073	1.177	0.959	1.049
	O	−0.250	−0.342	−0.397	−0.442	−0.641	−0.772	−0.572	−0.685
	HN	0.365	0.389	0.281	0.294	0.407	0.446	0.377	0.418
	C(C)	−0.050	−0.014	−0.133	−0.118	−0.114	−0.078	−0.115	−0.085
	C(N)	0.162	0.188	0.179	0.183	0.286	0.329	0.239	0.285
	μSCF	3.87	4.89	2.99	3.52	4.76	6.19	4.11	5.36
	μELE	3.01	4.19	3.00	3.54	4.77	6.19	4.14	5.41
TSanti	N	−0.570	−0.595	−0.739	−0.758	−1.072	−1.146	−0.894	−0.963
	C(O)	0.489	0.527	0.839	0.862	1.159	1.261	1.017	1.095
	O	−0.303	−0.381	−0.403	−0.433	−0.672	−0.789	−0.603	−0.697
	HN	0.313	0.332	0.292	0.303	0.441	0.476	0.385	0.417
	C(C)	−0.033	−0.005	−0.165	−0.158	−0.158	−0.139	−0.135	−0.120
	C(N)	0.104	0.121	0.175	0.184	0.301	0.337	0.231	0.264
	μSCF	1.62	2.30	0.60	0.78	2.48	3.30	1.87	2.49
	μELE	1.64	2.40	0.61	0.79	2.51	3.34	1.92	2.56
trans minima	N	−0.454	−0.451	−0.656	−0.670	−0.686	−0.693	−0.589	−0.598
	C(O)	0.412	0.434	0.808	0.832	0.899	0.966	0.768	0.816
	O	−0.325	−0.423	−0.429	−0.470	−0.697	−0.834	−0.607	−0.722
	HN	0.321	0.351	0.293	0.309	0.346	0.373	0.326	0.355
	C(C)	−0.048	−0.019	−0.172	−0.162	−0.107	−0.082	−0.092	−0.066
	C(N)	0.093	0.109	0.159	0.161	0.244	0.269	0.193	0.214
	μSCF	3.52	4.65	2.34	2.79	4.37	5.81	3.94	5.27
	μELE	2.88	4.17	2.32	2.76	4.33	5.79	3.91	5.22
trans CS minima	N			−0.480	−0.482	−0.679	−0.686		
	C(O)			0.647	0.669	0.876	0.937		
	O			−0.413	−0.462	−0.694	−0.831		
	HN			0.248	0.262	0.342	0.373		
	C(C)			−0.122	−0.111	−0.087	−0.058		
	C(N)			0.120	0.124	0.242	0.266		
	μSCF			2.57	3.15	4.38	5.88		
	μELE			2.52	3.09	4.33	5.86		

MonteCarlo simulations give an increase of 2 kcal/mol in the isomerization barrier of DMA in water.

The ability of the MP4$^{\#}$/6-31G* calculations to reproduce the solvation of the different conformations of NMA is clear from the preceding discussion. It cannot be expected that methods like AM1 and STO-3G, which provide inaccurate values of rotational barriers in vacuum, will perform correctly in water. Nevertheless, inspection of Table V reveals that the lower level calculations, except the STO-3G, provide good $\Delta\Delta G$ of solvation at least qualitatively, which confirms the suitability of the cavity selected. The good behavior of the SCRF-AM1 algorithm is encouraging, since it provides an inexpensive method for representing solvation phenomena qualitatively.

Changes in Charge Distribution. The combined analysis of the dipole moment, electrostatic charges and the MEP provides a complete picture of the molecular charge distribution. As noted in the Methods section, a preliminary study aimed at showing the reliability of the procedure to obtain charges was performed. For this purpose, electrostatic charges and dipoles for the four conformers of NMA were computed at the AM1 level by using two layers with a density of points ranging from 0.5 point/Å^2 to 10 points/Å^2 with increments of 0.5 point/Å^2. These calculations are a tedious task, but they are an excellent test of the robustness of the charges. Analysis (plots are available from the authors upon request) of the average and maximum differences for charges, and of the differences for electrostatic dipoles derived at densities i and $i + 0.5$ shows that errors are small for densities up to 1 point/Å^2 and almost negligible for densities up to 6 points/Å^2. Additional tests guaranteed accuracies in the range of the thousandth of electron in charges for a density of 10 points/Å^2. Consequently, all the fittings were performed using this value, which allows significant charge differences to be considered.

Electrostatic charges and dipole moments in both gas-phase and aqueous environments are shown in Table VII, where the SCF dipole moment is also reported. These charges, despite the criticisms shared by other scalar charges,[8c,46,47] properly reproduce the charge distribution

Table VIII. Absolute (D) and Relative (%) Differences between SCF (Roman Text) and Electrostatic (Italics Text) Dipoles in Vacuum and Aqueous Solution

	AM1	STO-3G	6-31G	6-31G*
cis	1.28 (33)	0.46 (20)[a] 0.59 (22)	1.52 (31)	1.34 (32)
	1.42 (43)	*0.45 (19)* *0.58 (21)*	*1.55 (32)*	*1.36 (32)*
TSsyn	1.02 (26)	0.53 (18)	1.43 (30)	1.26 (31)
	1.18 (39)	*0.54 (18)*	*1.41 (30)*	*1.27 (31)*
TSanti	0.71 (43)	0.18 (30)	0.82 (33)	0.62 (33)
	0.76 (46)	*0.17 (28)*	*0.83 (33)*	*0.64 (33)*
trans	1.13 (32)	0.45 (19)[a] 0.58 (23)	1.44 (33)[a] 1.50 (34)	1.33 (34)
	1.29 (44)	*0.44 (19)* *0.57 (23)*	*1.46 (34)* *1.53 (35)*	*1.31 (34)*

[a] Values for the C1 (top) and CS (bottom) conformers.

and the reactive characteristics of the molecules.[10i,25,26a,b,46,48] The agreement between SCF and electrostatic dipoles for all the basis sets supports the reliability of these charges when (i) they are obtained from suitable geometries and (ii) when the fitting is performed in an adequate set of points. All the methods give qualitatively similar values, but there are numerical differences. Thus, AM1 and the STO-3G basis underestimate the charges (in absolute values) with respect to the 6-31G*, whereas the 6-31G basis overestimates them.

The 6-31G* basis correctly describes the electrostatic distribution of molecules.[23,24] At this level the dipole moments for NMA in the gas phase are 3.94 (*trans*) and 4.20 (*cis*) D, which are close to the values reported by Wolfenden and co-workers[38] at the 6-31G** level (4.03 (*trans*) and 4.21 (*cis*) but also agree with the experimental range of dipole moments for NMA (3.85–4.22 D) in highly apolar solvents ($\epsilon = 2$).[45] Table VII shows that even AM1 or 6-31G wavefunctions give satisfactory descriptions.

Electrostatic charges for *cis* and *trans* are slightly different (around 0.02–0.05 electrons), and the most relevant changes appear when planar and twisted conformers are compared. The largest change is detected at the nitrogen, which increases its negative charge by more than 0.3 electrons at the 6-31G* level in the twisted form, its nucleophilicity being enhanced. The electrophilicity of the carbonyl carbon is also increased (around 0.2 electrons). The amide hydrogen becomes slightly more acidic (around 0.05 electrons). Changes in the charges of the methyl groups partially compensate for variations in the charges of nitrogen and carbonyl. Thus, the methyl group bound to the carbon enlarges its negative charge by a maximum of 0.1 electrons, whereas the other methyl group becomes at most 0.1 electrons more positive in the twisted conformers. Finally, oxygen of the TSsyn is less negative than those of planar structures, but in the TSanti it has a negative charge similar to that found in the *trans* conformation.

The effect of water, although less drastic than that of the conformation, is far from negligible. Water increases the charge separation, since in general charges are enhanced. This is stated in Table VIII, where increases of up to 1.3 D in the dipoles are found (variations of up to the 30% in the dipole). All the methods, except the STO-3G, give a similar variation. The oxygen is the most sensitive atom to charge variations by the solvent because its charge increases by more than 0.1 electrons for all conformations. The carbonyl carbon enlarges its positive charge, which increases the C–O bond dipole. Nevertheless, such a variation is not as large as that found for the oxygen (typically around 0.06 electrons). Similar behavior is shown by the amide hydrogen. Water has a small effect on the methyl group. Finally, the charge in the nitrogen remains unaffected by water in planar conformations, whereas in twisted conformers it increases by about 0.1 electrons.

Table IX. MEP Minimum Energy (kcal/mol) at the Nitrogen for the Different Conformations of NMA in Gas-Phase (Roman Text) and Aqueous Solution (Italic Text) Computed from the AM1, STO-3G, 6-31G, and 6-31G* Wave Functions

	AM1	STO-3G[a]	6-31G[a]	6-31G*
cis	−53.4	−21.9	−14.0	−12.9
	−49.9	*−20.3*	*−10.7*	*−11.1*
TSsyn	−115.7	−93.1	−86.6	−70.4
	−124.8	*−96.7*	*−95.7*	*−80.5*
TSanti	−104.5	−88.7	−71.7	−62.0
	−104.8	*−89.7*	*−70.6*	*−62.9*
trans	−54.5	−21.5	−12.6	−11.3
	−49.9	*−19.3*	*−7.2*	*−7.5*

[a] Values for the CS conformer.

Additional information can be derived from the MEP, which is the expectation value of the $\mathbf{r}^{-1}$ operator, and accordingly it is not subject to the uncertainties of any charge analysis. The MEP has several singular points,[49] the minima being the most informative about the nucleophilic characteristics of an atom. We computed the MEP minimum corresponding to the amide nitrogen in the N lone pair minima for the different conformations[26c] (Table IX). A large increase in the depth of the MEP minimum upon twisting of the amide bond is found (up to 50 kcal/mol.[50]) For intance, at the 6-31G* level the minimum changes from −12.9 kcal/mol (*cis*) or −11.3 kcal/mol (*trans*) to −70.3 kcal/mol (TSsyn) and −62.0 kcal/mol (TSanti). This variation agrees with the trends shown by the electrostatic charges and supports the enhanced nucleophilicity/electrophilicity of the nitrogen/carbonyl carbon, respectively.

As reported by other authors,[7,8,10h,i,k] conformation largely influences the charge distribution of the peptide bond, and specifically of the NMA, which is reflected in major changes in the dipole. Results in Tables VII–IX cannot fully support Pauling's classical resonance scheme,[7] since the charge in the oxygen remains almost unchanged throughout the rotation. In addition to the small change in the C–O distance, this suggests a lower involvement of the oxygen in the twisting than that predicted from Pauling's scheme. A similar effect has been reported in different resonance structures using both electrostatic and Bader's[51] charges.

Results suggest changes in the nucleophilicity/electrophilicity of the NMA upon rotation that agree better with an electron transfer in the direction C(O) → N. Nevertheless, a direct comparison between electrostatic and Bader's charges cannot be performed.[26c,8c,51] Electrostatic charges are expected to represent the MEP distribution around the molecule "isotropically". Bader's charges,

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(50) Planar conformations of NMA have two equivalent N-minima, whereas twisted structures have only one. Nevertheless, even multiplying the N-minima depth of planar structures by a factor of 2, the differences are very large in the range of 40–50 kcal/mol.

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which are derived from the integration of the electron density, need higher order atomic multipoles to represent the MEP distribution. Therefore, an increase in Bader's negative charge at the C(O) and positive charge at the N during the amide rotation do not necessarily mean an increase in the electrophilicity of N or an increase in the nucleophilicity of C when the amide bond is rotated.

To gain insight into the changes in charge distribution, we computed the "global" charges, which were obtained by adding the electrostatic charges of the atoms at each side of the C–N bond. They are expected to give an average "anisotropic" picture of the changes in nucleophilicity/electrophilicity. From these charges, an increase around 0.2 electrons in the electrostatic charge of the N-side was detected, which agrees with an enhancement of the nucleophilic character of the N-side and a parallel increase in the electrophilic character of the C-side upon rotation.

The above results reflect a large range of experimental results, like the accelerated hydrolysis of twisted amides,[52] or the lowering in the barrier at low pH.[40a,53] They also agree with the effect of substituents on the rotational barrier of DMA, like the increase in the barrier when the *C*-methyl group is substituted by a fluorine or by azido groups.[1n] In addition, structure–activity relationships reveal that a methyl group at the carbonyl side decreases the rotational barrier by 1 kcal/mol, while an increase of 1 kcal/mol is found if the methyl group is attached to the nitrogen side.[1h,j,n]

SCRF results (Tables VII–IX) show the relevant effect of water, which stabilizes polar structures.[10j] Results agree with *ab initio* data indicating that the oxygen has the strongest interaction with water[10a] and with the enlargement in the nucleophilicity of the nitrogen for twisted amides. Table VII also shows that differences of up to 0.1 electrons can be induced by water. Gao and Jorgensen[10h] reported that for NMA errors in charges of only 0.07 electrons lead to misleading results in MonteCarlo simulations. Therefore, since charge parametrization in previous studies[10h,i] was performed in vacuum, the agreement found between the reported $\Delta\Delta G$ of solvation and experimental data may be surprising. Nevertheless, in those studies the polarization effect of water was introduced in the parameters used for the simulation, since charges[10h] or van der Waals parameters[10i] were fitted in such a way that the *ab initio* interaction energy between water molecules and the NMA was reproduced.

Present results provide a complete picture of the energetics of the *cis–trans* isomerization of NMA in both the gas-phase and aqueous solution. Moreover, the use of SCRF theory, and the analysis of the dipole and MEP distribution, make it possible to gain insight into the changes in the electrostatic properties of NMA originating from the conformation and the environment. Energetics at the $\mathrm{MP4}^{\#}$/6-31G* level compare well with available experimental data in both vacuum and aqueous solution. Water preferentially stabilizes the TSsyn, but not enough to shift the *in vacuo* preference for the TSanti. In fact, the net effect of water is the increase in the barrier, since the minimum conformations are better solvated than the most stable transition state. This agrees with recent suggestions[37a] indicating that the apolar environment of peptidyl-prolyl isomerases plays a key role in facilitating the rotamase action. Nevertheless, since the expected stabilization is only 3 kcal/mol, other specific interactions must exist at the active site to explain their large catalytic ability.[37,54]

Conclusions

The rotation of amides is a highly unfavored process irrespective of the environment, as noted by the barriers of 17.68 and 20.13 kcal/mol computed at the $\mathrm{MP4}^{\#}$/6-31G* level for the *trans* ↔ *cis* isomerization in vacuum and in aqueous solution. The *anti* transition state is more stable than the *syn* by a difference of 4.2 kcal/mol in the gas phase and 1.2 kcal/mol in water. Water has only a moderate influence on the *cis/trans* equilibrium but a major effect on the kinetics of isomerization. Thus, the rotational barrier is increased by 2.5 kcal/mol in water. Such an increase stems from the greater stabilization of planar structures with respect to the *anti* transition state.

The nitrogen becomes a better nucleophile, and the carbon a better electrophile, when the amide bond is twisted. Water has an important effect in determining the electrostatic distribution of molecules in solution, as shown by the variations of up to 30% in the molecular dipole. The oxygen atom is the most sensitive to the presence of water, which produces an increase of around 0.1 electrons in its negative charge.

Comparison between different computational methods indicates that 6-31G* is the minimum level necessary to study the amide rotation, although correlation corrections must be introduced to obtain quantitatively correct values. The STO-3G and 6-31G basis sets and the semiempirical AM1 are inadequate to study the energetics of amide twisting. Nevertheless, the 6-31G and the AM1 give qualitatively correct information about the solvent effect, as well as about the changes in electrostatic charge distribution occurring during the amide twisting. Tomasi's SCRF method, when combined with suitable descriptions of the cavity size and good SCF calculations, can lead to suitable representations of solvation phenomena.

Acknowledgment. This paper was written when the authors joined Prof. W. L. Jorgensen's group at Yale (M.O.) and Prof. W. F. van Gunsteren's group at the E.T.H. (F.J.L.). We are very grateful for their hospitality. We are also indebted to Drs. J. Tirado-Rives, D. Severance, and E. Duftin for many helpful suggestions. We thank Drs. S. Olivella and J. M. Bofill for making available their personal version of MOPAC, which was modified to perform solvation calculations, and Prof. J. Tomasi for his version of Monstergauss. This work has been supported by the Comissió Interdepartamental de Recerca i Innovació Tecnológica (CIRIT QFN91-4203), by the Centre de Supercomputació de Catalunya (CESCA), and by the Chemical Structure Association.

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