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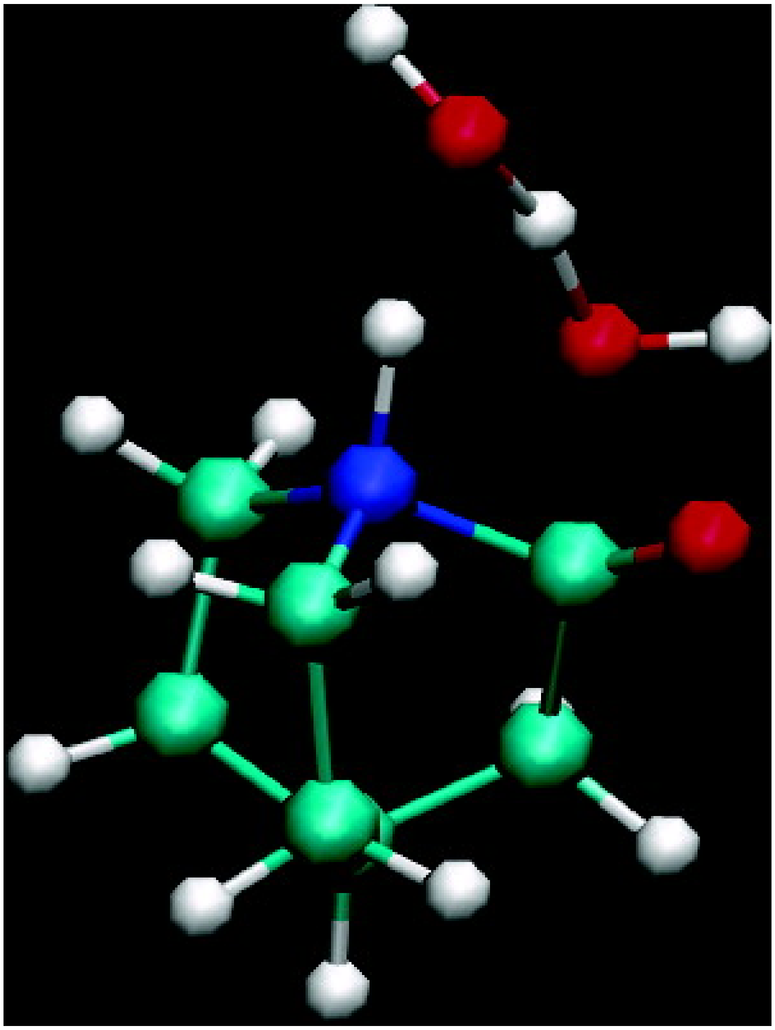

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# Water-Promoted Hydrolysis of a Highly Twisted Amide: Rate Acceleration Caused by the Twist of the Amide Bond

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***Abstract:*** The water-promoted hydrolysis of a highly twisted amide is studied using density functional theory in conjunction with a continuum dielectric method to introduce bulk solvent effects. The aim of these studies is to reveal how the twisting of the C−N bond affects the neutral hydrolysis of amides. To do so, both concerted and stepwise mechanisms are studied and the results compared to the ones from the hydrolysis of an undistorted amide used as reference. In addition, an extra explicit water molecule that assits in the required proton-transfer processes is taken into account. Our results predict important rate accelerations of the neutral hydrolysis of amides when the C−N bond is highly twisted, the corresponding barrier relaxation depending on the specific reaction pathway and transition state involved. Moreover, our calculations strongly suggest a change in reaction mechanism with degree of amide bond twist, and clearly point to a concerted mechanism at neutral pH for the hydrolysis of highly twisted amides. In addition, the twisting of the amide bond also provokes a higher dependence on an auxiliary water molecule for the concerted mechanism, due to the orthogonality of the lone pair of the nitrogen and the carbonyl $\pi$ orbital. There is a direct implication of these findings for biological catalytic mechanism of peptide cleavage reactions that undergoes ground-state destabilization of the peptide.

## 1. Introduction

The amide bond is the essential structural motif of the protein backbone. The hydrolysis reaction of amides, often used as a model for the cleavage of peptide bonds,[1,2] is thus of primary concern for living systems. The water-promoted hydrolysis, or neutral hydrolysis, of nonactivated amides is very slow and in most cases undetectable.[3] The half-life for the hydrolysis of acetyl-glycil-glycine is 500 years[4] at pH 6.8 and 25 C. An amide bond's stability is ascribed to its partial double bond character, caused by the delocalization between the nitrogen lone pair and the $\pi_{CO}$bond.[5] As a consequence, amides show a characteristic short C−N bond length and a rigid planar conformation. This stability also has important chemical consequences, a low reactivity toward nucleophilic attacks on the carbon and important basicity shifts of the nitrogen with respect to amines.[6,7]

A hypothesis for activation of amides toward hydrolysis establishes that the amide bond could be significantly weakened by twisting the amide bond in the reactant, and thus, imposing orthogonality between the nitrogen lone pair and the carbonyl $\pi$-system. This would destabilize the reactant by breaking the $n_N \rightarrow \pi^*_{CO}$ delocalization and lead to the nitrogen pyramidalization followed by a C−N bond lengthening.[6,7] The twist of the amide bond could be induced by specific geometrical constraints or by enzyme action on a preferential twisted conformation of the substrate. For example, this type of "ground-state destabilization" has been suggested to be part of the mechanism of catalysis in the case of protein splicing.[8,9] This is as well the basis for the design of transition state analogues which are used as haptens to develop catalytic antibodies[10] that are able to catalyze peptide bond cleavage by inducing "ground-state detabilization" of the reactant. Furthermore, nonplanar deformations of amide bonds in peptides have been the focus of considerable interest.[11]

X-ray data[12] and computational studies[13−19] on the rotational barrier of formamides and its derivatives support the idea of

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C−N bond weakening upon C−N bond twist. Unfortunately, little accurate kinetic data on hydrolysis of twisted amides is available. However, in a seminal work by Blackburn et al., it was reported that the alkaline hydrolysis of benzoquinuclidin-2-one, a strained cyclic amide with a significantly twisted amide bond, was $10^7$ times faster than its strainless counterpart.[20,21] This corresponds to a decrease in the reaction activation energy by about 10 kcal/mol. In agreement with these experiments, Brown et al. also reported[2,22] significant accelerations for the hydrolysis of distorted amides, ranging from 7 orders of magnitude for the alkaline hydrolysis to 11 orders of magnitude for acid-catalyzed hydrolysis. More recently, Kirby et al.[23−25] also reported the rapid hydrolysis in water (under slight acidic conditions) of a highly twisted amide bond in 1-aza-2-adamantanone, suggesting an even higher acceleration of the hydrolysis rate.

Computational studies[26−42] on the hydrolysis of amides have been mainly focused on the reaction of undistorted planar amides such as formamide. The specific question of how the twisting of the amides affects its properties was early studied by Greenberg et al.[6,43] They found that in the gas phase and contrary to behavior of undistorted amides, highly twisted amides are protonated at the nitrogen instead of at the oxygen, which suggested important mechanistic differences for the hydrolysis of twisted amides in acidic and neutral medium. More recently, we characterized[7] the p$K_a$ in solution for a series of twisted amides, which confirmed the results of Greenberg et al., and revealed a p$K_a$ of 6−7 for the most twisted amides. In a recent paper,[44] we made a comparative computational study of the alkaline hydrolysis of a twisted amide (**TA** in Figure 1) with respect to its planar analogue (**PA** in Figure 1), asserting a significant rate acceleration for the stepwise mechanism with differential energy barriers between the two hydrolysis of 7.0−9.7 kcal/mol in the case of the transition state for the nucleophilic attack of OH− on the carbonyl carbon, which showed good agreement with the experimental estimates.

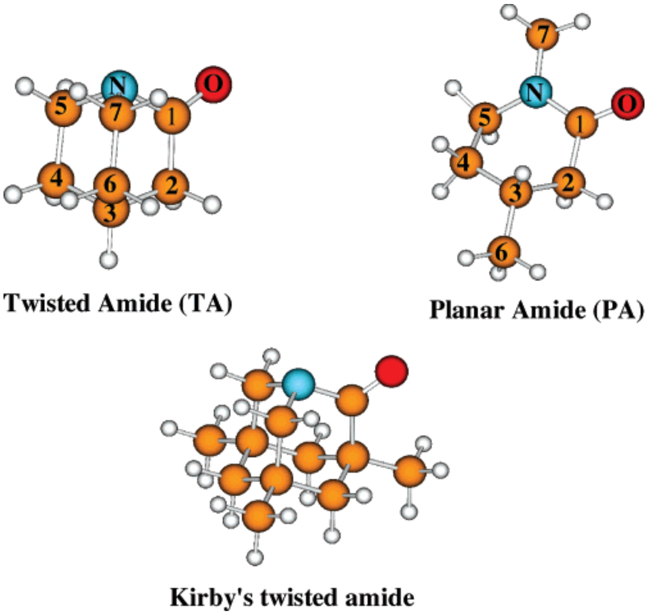

***Figure 1.*** (Top) The two amide reactants used along the present study. (Left) 1-Aza-bicyclo[2.2.2]octan-2-one twisted amide is shown (**TA**), and on the right-hand side the planar amide analogue 1,4-Dimethyl-piperidin-2-one (**PA**). At the bottom of the figure Kirby's most twisted amide 3,5,7-Trimethyl-1-Aza tricycle[3.3.1.1$^{1,5}$] decan-2-one.[23,24]

In the present paper, the water-promoted hydrolysis of a highly twisted amide (**TA** in Figure 1) is studied in detail. This regime is crucial to understand the hydrolysis of this kind of amides under neutral values of pH, and hence, it is most relevant under biological conditions. Both concerted and stepwise mechanisms are considered and the energetic barriers compared to the ones obtained for a reference undistorted planar amide analogue (**PA** in Figure 1). In all mechanisms, an additional water molecule is also considered, which catalyzes the reaction by assisting in proton transfer. Density functional theory is used for this purpose, in conjunction with polarizable continuum models[45] to take into account bulk solvent effects. Our computational studies reveal important differences between the neutral hydrolysis of **TA** and **PA**. Substantial rate-accelerations are predicted but more importantly a clear preference for a concerted mechanism is observed for the hydrolysis of **TA**. Our calculations also reveal that the rate accelerations obtained in neutral hydrolysis are significantly higher than the ones obtained for the alkaline hydrolysis, motivated in part by the preference on a concerted mechanism. The results presented here are thus fundamental to understand the rate acceleration caused by the twisting of the C−N bond.

## 2. Methodology

Potential energy surfaces for the neutral hydrolysis of a twisted amide and a planar amide (**TA** and **PA** in Figure 1) were characterized in the gas phase and in solution. All the calculations were carried out using the Gaussian 98 suite of programs.[46] Gas-phase structures were optimized at the B3LYP/6-31+G(d) level of theory. Then, single-point B3LYP/6-311++G(d,p) calculations were done at these geometries to

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***Table 1.*** Relative Electronic Energies ($\Delta E_e$), Zero-Point Energies ($\Delta E_0$), Enthalpies ($\Delta H$), Entropic Contributions ($T{\cdot}\Delta S$) and Free Energies ($\Delta G$) for the Stationary Points Involved in the Neutral Hydrolysis Reaction of the Twisted Amide in Gas-Phase and in Solution (values presented in bold)

| | $\Delta E_e$ | $\Delta E_0$ | $\Delta H$ | $T{\cdot}\Delta S$ | $\Delta G$ | $\Delta\Delta G_{as}$[a] |
|---|---|---|---|---|---|---|
| | | | concerted mechanism | | | |
| react | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** |
| $TS_c$ | 17.4 | 17.9 | 15.9 | −16.8 | 32.7 | −13.6 |
| | **20.7** | **21.3** | **19.6** | **−15.9** | **35.5** | **−12.1** |
| prod | −19.0 | −14.4 | −15.6 | −14.2 | −1.4 | 0.0 |
| | **−12.4** | **−8.0** | **−9.3** | **−14.6** | **5.4** | **1.2** |
| | | | stepwise mechanism | | | |
| $TS1_s$ | 21.9 | 22.3 | 19.8 | −17.4 | 37.2 | −11.4 |
| | **28.7** | **28.8** | **26.4** | **−17.4** | **43.8** | **−10.0** |
| $Int_s$ | −9.6 | −4.8 | −6.6 | −15.8 | 9.2 | −1.5 |
| | **−1.2** | **3.3** | **1.6** | **−15.8** | **17.4** | **0.8** |
| $TS2_s$ | 1.8 | 5.1 | 2.7 | −17.1 | 19.8 | −24.3 |
| | **8.3** | **11.6** | **9.3** | **−17.1** | **26.4** | **−22.2** |

[a] $\Delta\Delta G_{as}$ comes from the catalytic effect provided by the assistant/auxiliary water molecule, defined according to formula 4. All numbers are in kcal/mol.

re-evaluate the electronic energy, which will then be used to compute the relative energy with respect to the separate reactants. The use of the B3LYP functional[47−50] is motivated by its success in the evaluation of reliable reaction enthalphies for the hydrolysis of neutral amides.[33] The enthalpic and entropic corrections were determined with a frequency calculation at B3LYP/6-31+G(d) level of theory.[51] These frequencies were also used to verify the nature of the stationary points encountered along the potential energy surface. Thus, reactants, intermediates and products showed real frequencies for all the normal modes of vibration, whereas transition states showed one imaginary frequency along the normal mode that connects the appropiate minima. The frequency calculations were also used to allow evaluation of thermodynamic quantities such as the zero-point vibrational energy, and thermal vibrational contributions to the enthalpy, entropy and Gibbs free energy.

All structures were also optimized in solution using the Self-Consistent Isodensity Polarizable Continuum Model (SCI-PCM) method[52] at the B3LYP/6-31+G(d) level of theory. The same level of theory was used for the frequency calculations, and the electronic energy in solution was calculated at the B3LYP/6-311++G(d,p) level of theory. The value of the electron density used to describe the cavity's size and shape into which the solute will be placed was set to 0.0004 au, and 146 grid points were considered for the surface charges.

The gas phase and solution energies were employed to calculate the relative energies with respect to the separate reactants: amide + water and amide+water-dimer. The relative energies are collected in Tables 1 and 3.

The nomenclature of the atoms in the various reaction mechanisms are summarized in Figure 2. The $C_1$ and O atoms correspond to the carbonyl atoms in the amide. The $O_{nu}$ atom stands for the oxygen of the water molecule that makes the nucleophilic attack on the carbonyl $C_1$ atom. The oxygen atom of the water molecule that assists the hydrolysis is called $O_{as}$, and the protons to transfer are named $H_t^{nu}$ and $H_t^{as}$. The nu and as superscript refer to the bonding of these protons to $O_{nu}$ or $O_{as}$ at the start of the reaction.

The out-of-plane deformations are described by the angles $\tau$, $\chi_C$[12,53] and $\chi_N$. The angle $\tau$ characterizes the mean twisting angle around the $C_1-N$ bond and ranges from 0 (planar amide group) to 90 (when the two planes defined by the $O-C_1-C_2$ and $C_5-N-C_6$ atoms are perpendicular); $\chi_C$ and $\chi_N$ are measures of the degree of pyramidalization at the $C_1$ and N, respectively. They range approximately from 0 (planar $sp^2$ atoms) to 60 (tetrahedral $sp^3$ atoms). The four torsion angles $\omega_1 = O-C_1-N-C_5$, $\omega_2 = C_2-C_1-N-C_6$, $\omega_3 = C_2-C_1-N-C_5$ and $\omega_4 = O-C_1-N-C_6$, are combined to define the $\tau$, $\chi_C$ and $\chi_N$ as follows

$$\tau = \frac{(\omega_1 + \omega_2)}{2} \tag{1}$$

$$\chi_C = \omega_1 - \omega_3 + \pi(\mathrm{mod}2\pi) = -\omega_2 + \omega_4 + \pi(\mathrm{mod}2\pi) \tag{2}$$

$$\chi_N = \omega_2 - \omega_3 + \pi(\mathrm{mod}2\pi) = -\omega_1 + \omega_4 + \pi(\mathrm{mod}2\pi) \tag{3}$$

The absolute values for these angles with projection on the 0−90 quadrant are found in Table 2. In all cases, the oxygen atom used to calculate the $\omega_1$ and $\omega_4$ torsion angles corresponds to the original carbonyl oxygen rather than the water oxygen. For the product structures in which the amide bond is broken, these angles are of limited value. In those cases, we use the improper dihedrals.

## 3. Results

In this section, the main structural and energetic features of the characterized potential energy surfaces are presented. For the sake of brevity, we will only present in detail the structures corresponding to the water-assisted mechanism in solution for the hydrolysis of the twisted amide. Cartesian coordinates of all optimized structures, both in the gas phase and in solution, for both planar and twisted amide reactants are accessible as Supporting Information. The reaction mechanisms can be classified according to the number of steps involved (concerted vs stepwise). The *concerted* mechanism shows only one transition state (TS) between the reactants and the products, whereas in the *stepwise* mechanism there are two transition states involved with formation of a tetrahedral amide carbon intermediate: one that corresponds to the nucleophilic attack on the amide carbon (TS1) and another one related to the cleavage of the C−N amide bond (TS2). For both concerted and stepwise mechanisms, there are two water molecules taking part in the reaction, one as the nucleophile and the other as an auxiliary water molecule which catalyzes the hydrolysis by serving as a bridge for transferring protons. The effect that this auxiliary water molecule has on the reaction barriers was evaluated as well, by removing the water from the mechanism and re-characterizing the transition states.

To facilitate the identification of the transition states and intermediates for each reaction we will introduce a nomenclature to specify each stationary point. The subscripts c and s will specify whether the structure belongs to the concerted (c) or stepwise (s) mechanism. For example, $TS1_s$ and $TS2_s$ will be

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***Table 2.*** B3LYP/6-31+G* Geometrical Parameters for the Stationary Points of the PES's of the Water-Assisted Neutral Hydrolysis of Twisted Amide Following the Concerted and Stepwise Mechanisms[a]

Concerted Mechanism

| | bond distances | | | | | | | | angles | | | improper dh. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $C_1$–N | $C_1$–O | $C_1$–$O_{nu^-}$ | N–$H_t^{as}$ | $O_{as}$–$H_t^{as}$ | $O_{as}$–$H_t^{nu}$ | $O_{nu^-}$–$H_t^{nu}$ | - | $\tau$ | $\chi_c$ | $\chi_n$ | $dh_C$ | $dh_N$ |
| react | 1.453 | 1.209 | | | | | | | 89.4 | 0.1 | 62.4 | 180 | 117.2 |
| | **1.448** | **1.216** | | | | | | | **89.9** | **0.0** | **62.8** | **180.0** | **116.7** |
| $TS_c$ | 1.553 | 1.210 | 2.139 | 1.121 | 1.474 | 1.257 | 1.189 | | 36.0 | 30.8 | 60.3 | 146.0 | 116.5 |
| | **1.533** | **1.209** | **2.348** | **1.076** | **1.641** | **1.200** | **1.247** | | **30.9** | **23.8** | **60.6** | **153.2** | **116.9** |
| Prod | 3.631 | 1.217 | 1.349 | 1.018 | 3.215 | 0.998 | 2.775 | | 44.4 | 55.7 | 55.7 | 176.5 | 121.3 |
| | **3.512** | **1.223** | **1.346** | **1.019** | **3.128** | **1.005** | **2.719** | | **44.6** | **29.7** | **42.2** | **177.6** | **120.0** |

Stepwise Mechanism

| | bond distances | | | | | | | | angles | | | improper dh. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $C_1$–N | ($C_1$–O) | $C_1$–$O_{nu^-}$ | N–$H_t^{nu}$ | $O_{as}$–$H_t^{nu}$ | $O_{nu^-}$–$H_t^{nu}$ | $O_{as}$–$H_t^{as}$ | O–$H_t^{as}$ | $\tau$ | $\chi_c$ | $\chi_n$ | $dh_C$ | $dh_N$ |
| $TS1_s$ | 1.451 | 1.299 | 1.771 | 2.770 | 1.219 | 1.228 | 1.166 | 1.290 | 35.3 | 43.1 | 61.5 | 136.8 | 117.0 |
| | **1.465** | **1.312** | **1.691** | **2.792** | **1.234** | **1.210** | **1.141** | **1.324** | **35.7** | **45.7** | **62.1** | **134.3** | **116.9** |
| $Int_s$ | 1.484 | 1.397 | 1.426 | 1.903 | 0.992 | 2.833 | 1.926 | 0.987 | 26.1 | 59.6 | 60.6 | 120.4 | 119.8 |
| | **1.484** | **1.406** | **1.419** | **1.878** | **0.995** | **2.875** | **1.982** | **0.985** | **25.3** | **59.9** | **61.0** | **120.1** | **119.7** |
| $TS2_s$ | 1.671 | 1.340 | 1.385 | 1.048 | 1.792 | 2.540 | 1.368 | 1.119 | 27.9 | 58.2 | 56.3 | 121.8 | 122.7 |
| | **1.626** | **1.355** | **1.396** | **1.039** | **1.925** | **2.553** | **1.373** | **1.115** | **27.1** | **57.8** | **56.9** | **122.3** | **122.9** |
| Prod | 3.631 | 1.217 | 1.349 | 1.018 | 3.215 | 4.542 | 0.998 | 3.352 | 44.4 | 55.7 | 55.7 | 176.5 | 121.3 |
| | **3.512** | **1.223** | **1.346** | **1.019** | **3.128** | **4.455** | **1.005** | **3.352** | **44.6** | **29.7** | **42.2** | **177.6** | **120.0** |

[a] The bond distances are in Å and the angles and improper dihedrals in degrees for the structures optimized in Gas-Phase and in Solution (in bold).

***Table 3.*** Relative Electronic Energies ($\Delta E_e$), Zero-Point Energies ($\Delta E_0$), Enthalpies ($\Delta H$), Entropic Contributions ($T\cdot\Delta S$) and Free Energies ($\Delta G$) for the Stationary Point Involved in the Neutral Hydrolysis Reaction of the Planar Amide in Gas-Phase and in Solution (values presented in bold)

| | $\Delta E_e$ | $\Delta E_0$ | $\Delta H$ | $T$–$\Delta S$ | $\Delta G$ | $\Delta\Delta G_{as}$ |
|---|---|---|---|---|---|---|
| Concerted Mechanism | | | | | | |
| react | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** |
| $(TS_c)_{exo}$ | 34.8 | 34.6 | 32.8 | −15.6 | 48.3 | −6.0 |
| | **39.9** | **40.0** | **38.3** | **−15.3** | **53.6** | **−2.7** |
| $(TS_c)_{endo}$ | 35.2 | 35.2 | 33.3 | −16.1 | 49.3 | −4.2 |
| | **40.7** | **40.7** | **39.0** | **−15.5** | **54.5** | **−2.2** |
| prod | 2.8 | 6.5 | 5.3 | −13.8 | 19.1 | 0.4 |
| | **10.3** | **13.8** | **12.6** | **−13.6** | **26.2** | **1.7** |
| Stepwise Mechanism | | | | | | |
| $(TS1_s)_{exo}$ | 27.9 | 28.6 | 26.9 | −14.8 | 41.6 | −9.3 |
| | **34.8** | **36.3** | **34.6** | **−15.2** | **49.8** | **−5.3** |
| $(TS1_s)_{endo}$ | 25.5 | 25.8 | 23.9 | −15.9 | 39.8 | −8.6 |
| | **33.1** | **34.2** | **32.4** | **−15.6** | **48.0** | **−4.9** |
| $Int_s$ | 12.1 | 15.7 | 14.1 | −15.3 | 29.5 | 0.4 |
| | **20.4** | **23.8** | **22.3** | **−15.2** | **37.5** | **2.5** |
| $(TS2_s)_{exo}$ | 25.5 | 26.9 | 24.4 | −17.5 | 41.9 | −18.3 |
| | **31.1** | **33.4** | **30.8** | **−17.7** | **48.5** | **−15.8** |
| $(TS2_s)_{endo}$ | 22.8 | 25.6 | 23.0 | −17.6 | 40.7 | −17.0 |
| | **30.4** | **32.8** | **30.4** | **−17.4** | **47.8** | **−14.3** |

[a] $\Delta\Delta G_{as}$ comes from the catalytic effect provided by the assistant/auxiliary water molecule, defined according to formula 4. All numbers are in kcal/mol.

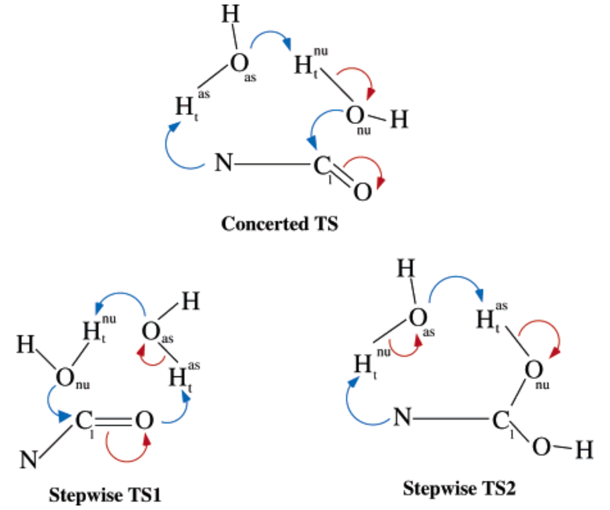

***Figure 2.*** Nomenclature used along the manuscript for the relevant atoms in the transition states.

used to name the first and second transition states in the stepwise mechanism, and $TS_c$ will refer to the transition state of the concerted mechanism.

**3.1 Water-Assisted Hydrolysis of the Twisted Amide. 3.1.1 Structures.** Selected geometrical parameters can be found in Table 2 and the structures are shown in Figure 3. The values in bold corresponds to the geometries optimized in solution using a SCI−PCM continuum model. The reactant presents an amide bond with a significant degree of twist, enforced by the cage structure of the molecule. As it can be seen in Table 2, the twist measured by the $\tau$ angle is 89.9, that is almost 100% degree of twist. Hence, the carbonyl $\pi_{C_1-O}$ orbital and the nitrogen's lone pair are in perpendicular planes, and therefore, there is no $n_N \rightarrow \pi_{C_1\text{-}O^*}$ delocalization. This leads to the loss of the partial double bond character of the $C_1$−N bond, which implies a pyramidalization of the nitrogen ($\chi_N$ is 62.8), and $C_1$−N bond lengthening compared to untwisted amide bonds. In particular, the $C_1$−N bond length in **TA** is 1.448 Å almost 0.1 Å longer than the one found in formamide at the same level of theory, and the C−O bond distance is 1.216 Å. As expected, the twist of the amide bond does not affect the carbonyl carbon planarity, $\chi_C$ is 0.0. The geometrical parameters of the amide unit in the reactant compares well with the X-ray values[23] reported for Kirby's most twisted amide, a trimethyl variant of 1-Aza-2-adamantone (See Figure 1). They also reported a very similar value of $\tau$, 90.5° and a C−N bond distance of 1.475 Å and a C−O one of 1.196 Å. Morgan and co-workers[54] have characterized 1-Aza-2-adamantone at B3LYP/6-31+G* level of theory and their values for the amide unit are also quite coincident with the values of our **TA** amide reactant, namely, 1.46 Å for

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(54) Morgan, K.; Rawlins, M.; Montgomery, M. *J. Phys. Org. Chem.* **2004**.

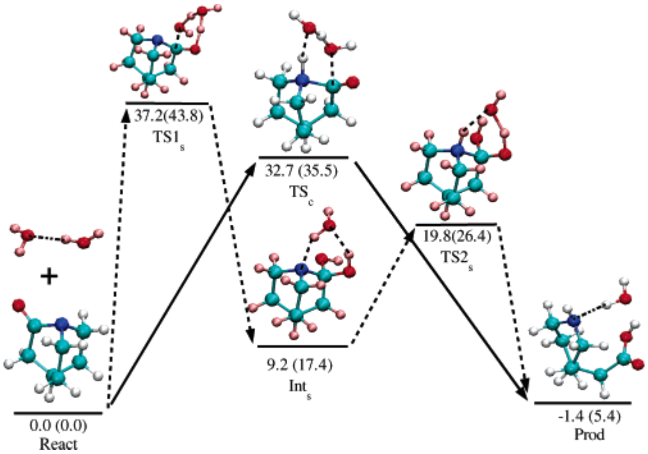

***Figure 3.*** B3LYP/6-31+G(d) structures for the twisted amide in the water-assisted hydrolysis reaction for both the concerted (regular-line) and stepwise (dashed-line) mechanism. The values correspond to the relative free energy ($\Delta G$) in kcal/mol, the first one for structures optimized in the gas phase, whereas the values in parentheses are for the structures optimized in solution. Note that the PES's are not scaled.

the C−N bond length and 1.21 Å for the C−O one. Therefore, although they are not the same molecule, our **TA** twisted amide and 1-Aza-2-adamantone show a very similar amidic subunit.

We have three transition states: one for the concerted mechanism TS$_c$, and two for the stepwise mechanism: TS1$_s$ and TS2$_s$. In all these transition states, an assistant water molecule is acting as a proton bridge, receiving a proton and donating one in turn (see nomenclature in Figure 2). We will now comment in detail each structure.

TS$_c$ is the transition state of the concerted reaction mechanism. The unique transition state of this mechanism corresponds to the nucleophilic attack of one of the water molecules on the amidic carbon (forming the $C_1$−$O_{nu}^-$ bond) the transfer of a proton from the nucleophilic water to the auxiliary water molecule, and in turn, a transfer of a proton from this water molecule to the nitrogen with cleavage of the amide bond. All of these geometrical changes happen in a concerted but asynchronous way. Whereas at the transition state the proton transfer to the nitrogen is almost completed (the N−$H_t^{as}$ distance is 1.076 Å), the $H_t^{nu}$ proton is almost equally shared by both water oxygens (the $H_t^{nu}$−$O_{as}$ and $H_t^{nu}$−$O_{nu}^-$ distance are 1.200 and 1.247 Å, respectively), and the attacking $O_{nu}$ is still at 2.348 Å from carbonyl carbon.

TS1$_s$ is the transition state that corresponds to the nucleophilic attack on the amidic carbon and intermediate formation. The $C_1$−$O_{nu}^-$ distance at the transition state is quite short, 1.691 Å significantly shorter than in the concerted mechanism (TS$_c$), 2.348 Å. The attack is concerted with a double proton transfer, one between water molecules ($H_t^{nu}$) and the other between the assistant water to the carbonyl oxygen ($H_t^{as}$). The degree of proton transfer is higher between the two water molecules than between the assistant water and the carbonyl oxygen. Thus, whereas $H_t^{nu}$ is almost equally shared by the two water oxygens (the $H_t^{nu}$−O distances are 1.234 and 1.210 Å), $H_t^{as}$ is still closer to the water than to the carbonyl oxygen (its distances with respect to $O_{as}$ and O are 1.141 and 1.324 Å, respectively).

After passing through TS1$_s$, the intermediate compound is formed. The proton transfer is completed, and correspondingly, the $H_t^{as}$−O distance is short, 0.985 Å. The carbonyl carbon is now forming two single C−O bonds with similar distances, namely, 1.406 and 1.419 Å for $C_1$−O and $C_1$−$O_{nu}^-$, respectively. Now the $C_1$ carbon presents a tetrahedral conformation and the $\chi_C$ angle is therefore 61.0. This conformational change makes the $C_1$−N bond less twisted with a decrease of the value of $\tau$ to 25.3. There is also a slight elongation of the $C_1$−N bond to 1.484 Å. The assistant water molecule is hydrogen bonded to the nitrogen atom and to one of the protons of the carboxilate group, and it is in a favorable position to serve again as a proton bridge in the second step of the reaction. An structure of a carbonyl hydrate intermediate at low pH has been characterized by Kirby et al.[24] in the case of the hydrolysis of a trimethyl variant of 1-Aza-2-adamantanone (see Figure 1). The values for the C−N and C−O bond distances for the crystal structure were 1.552 and 1.382 Å. Notice that at low pH both oxygen and nitrogen are protonated, which does not correspond to the intermediate characterized in the present work, and therefore, our C−N bond length is 0.7 Å shorter. When optimizing our intermediate with nitrogen protonated (low-pH or acid hydrolysis intermediate) we obtain values of 1.557 Å for the C−N bond length and 1.380 and 1.385 Å for $C_1$−O and $C_1$−$O_{nu}^-$ respectively, in quantitative agreement with the crystal structure of Kirby. This emphasizes that the low-pH intermediate is a stable species, and it is the protonation state detected by Kirby, whereas the neutral intermediate is difficult to form and if formed has a low barrier for breaking (see Discussion section).

The second transition state of the stepwise mechanism TS2$_s$ leads to the $C_1$−N bond cleavage. The $C_1$−N bond is quite activated at the transition state, 1.626 Å and it is almost 0.1 Å longer than in the concerted TS$_c$, 1.533 Å. The $H_t^{nu}$ proton is almost completely transferred to the nitrogen (the N−$H_t^{nu}$ bond distance is 1.039 Å). On the other hand, $H_t^{as}$ is still closer to O atom, 1.115 Å than to $O_{as}$, 1.373 Å. From these geometrical features and IRC analysis, we concluded that in the second step of the reaction, several changes occur in a concerted but asynchronous way. First, we have the proton transfer from the auxiliary water to the nitrogen, which then provokes the $C_1$−N bond cleavage and a proton transfer of the carboxilate to the assistant water molecule.

Finally, there is the product structure, which shows a carboxyl group with a $C_1$−$O_{nu}^-$ bond length of 1.346 Å, and a secondary amine with a N−H bond length of 1.019 Å. The $C_1$−N distance is 3.512 Å and there is a water molecule hydrogen bonded to the nitrogen.

**3.1.2 Energy Profile.** Gas-phase and solvent-phase relative energies with respect to the separated twisted amide and water dimer reactants can be found in Table 1. The relative energies are separated in its different contributions. We have negative gas-phase $\Delta E_e$ for the intermediate of the stepwise mechanism and the product of the hydrolysis, and positive $\Delta E_e$'s for all the transition states. The inclusion of ZPVE corrections increases the relative energies sligthly. The reaction is clearly exothermic in the gas-phase with a $\Delta H_{gas}$ value of $-15.6$ kcal/mol. Entropic contributions tend to increase the relative energies with respect to the separated reactants by 14−17 kcal/mol, as it corresponds to a loss of translational and rotational degrees of freedom when passing from two infinitely separate reactants to one single supramolecule. The relative free energy is therefore slightly negative in the gas-phase, $\Delta G_{gas}$ is $-1.4$ kcal/mol.

Regarding the transition states, in the concerted mechanism, the enthalpic barrier is 15.9 kcal/mol and free-energy barrier is

32.7 kcal/mol. On the other hand, the lowest-energy transition state in the gas-phase is $TS2_s$, with a $\Delta H_{gas}$ of only 2.7 kcal/mol and $\Delta G_{gas}$ of 19.8 kcal/mol. However, $TS1_s$ is the transition state of highest-energy ($\Delta H_{gas}$ is 19.8 kcal/mol and $\Delta G_{gas}$ is 37.2 kcal/mol). Therefore, in the stepwise mechanism, the first step is the rate-limiting one, and since, the barrier of this step is higher than the one of the concerted pathway, we can conclude that the concerted mechanism is the one preferred by the reaction in the gas phase.

Bulk solvent effects tend to stabilize the separated reactants, though by a small amount as compared to the alkaline hydrolysis.[44] Since all the stationary points in the PES are neutral, solvation free energies for each structure are small and are dominated by their volume (the smaller the volume the higher solvation free energy). This yields a solvation free energy for transition states, intermediates and products smaller in absolute value than the sum of the solvation free energies for the separated reactants. As a consequence, the relative energies for each stationary point will be larger in solution than in the gas phase. The exothermicity of the reaction is thus lower in solution, $\Delta H_{aq}$ is $-9.3$ kcal/mol and a positive value for $\Delta G_{aq}$ is obtained, namely, 5.4 kcal/mol. Nevertheless, the same clear mechanistic preference for the concerted mechanism as in the gas-phase is maintained, with an enthalpic barrier of 19.6 and 35.5 kcal/mol in free energy. With respect to the stepwise mechanism, the first step of the reaction ($TS1_s$) is clearly the rate-limiting step in solution as well, with a free energy barrier of 43.8 kcal/mol, much higher than the 26.4 kcal/mol free energy barrier of the second step ($TS2_s$).

**3.2 Hydrolysis of a Planar Amide Analogue as Reference.** To determine the effect that the twisting of the amide bond has on reaction barriers, we performed analogous calculations for the hydrolysis of a planar amide analogue to the twisted amide molecule of the previous section. The planar amide chosen is depicted in Figure 1 (**PA**), and it comes from the hydrogenation and cleavage of the **TA** $C_6-C_7$ bond. This leads to the breaking of the cage structure relieving the geometrical constraints that maintained the amide bond in a twisted conformation. The reactant now shows an untwisted amide bond with a corresponding low value of $\tau$, 1.8°, and a nitrogen atom in a quasi-planar conformation ($\chi_N = 8.4°$). This is the standard situation for an undistorted amide bond with some degree of delocalization between the lone pair of the nitrogen and carbonyl $\pi$ bond, leading to a partial double $C_1-N$ bond character. In fact, the $C_1-N$ bond length is shorter, 1.363 Å, than for the twisted amide reactant, 1.448 Å.

The energetic data can be found in Table 3. Due to the methyl group presence at the $C_3$ position, there will be two possibilities for TS1 and TS2 transition states, those that corresponds to attacks and proton transfers in an exo or an endo position with respect to this methyl group.

In general, both exo and endo reaction pathways give the same qualitative picture. Our energetic barriers are in reasonable qualitative agreement with previous calculations on the hydrolysis of formamides by other groups. In the context of nonwater-assisted mechanisms, Oie et al.[27] studied the neutral hydrolysis of the formamide using Moller−Plesset perturbation theory, for both concerted and stepwise mechanisms. They found that the energy barrier in the gas phase for the concerted mechanism at MP2/6-31G**//3-21G level of theory was 42.0 kcal/mol, whereas when they considered the stepwise mechanism, the approach of the water molecule required almost the same energy. The second step of the hydrolysis, that is, the peptide bond cleavage had a relative energy of 39 kcal/mol with respect to the reactants. Krug et al.[55] studied the neutral, acid and alkaline hydrolysis of the formamide at MP2(FULL)/6-31G**//4-31G level of theory. They found that the energy barrier for the concerted neutral hydrolysis was 44 kcal/mol. They also calculated the energy barrier for the stepwise mechanism, where the energy barrier for the first step was 42 and 35 kcal/mol for the second step. All of these values compare reasonably well with our $\Delta E_e$ values in the nonassisted mechanism, 42.0−43.0 kcal/mol for the concerted mechanism, and 38.0−40.4 kcal/mol for the first step of the stepwise mechanism. However, our values for the $TS2_s$ are somewhat larger, around 45 kcal/mol. This latter transition state is significantly stabilized in the water-assisted mechanism, obtaining similar gas-phase free energy barriers (39.8 kcal/mol for $TS1_s$ and 40.7 kcal/mol for $TS2_s$). Antonczak et al.[31,32] also looked at the role of an auxiliary water molecule for the neutral hydrolysis of formamide in the concerted mechanism. Using Moller−Plesset perturbation theory at Hartree−Fock geometries, they also determined that the effect of an auxiliary water molecule provides a moderate stabilization of the neutral transition state. Their MP3 values for the free energy barriers in solution were 59.3 kcal/mol for the nonassisted case and 56.7 kcal/mol for the water-assisted case, in good agreement with our values, 56−57 kcal/mol and 53.6 54.5 kcal/mol, respectively. In a more recent paper, Ruiz-López and co-workers[42] studied the amide bond formation in aqueous solution, the reverse reaction to the neutral hydrolysis of formamide, including static and dynamic solvent effects using a continuum dielectric method for the former and a QM/MM method for the latter. They investigated the nonassisted mechanism and they found that stepwise and concerted processes had similar activation free energies, 45.12 kcal/mol in the gas phase for the concerted mechanism and 45.75 kcal/mol for the stepwise mechanism, being the breakdown of the intermediate through a TS2 type transition state rate limiting, as it is our case for the nonassisted mechanism. From their results, it is concluded that both reaction mechanisms, concerted and stepwise compete in the case of the neutral hydrolysis of formamide.

Consequently, planar amides, opposite to highly twisted amides, hydrolyze under neutral pH conditions either through a stepwise or a concerted mechanism, depending on the specific chemical environment of the amide bond. For instance our calculations show that in the case of **PA**, there is a preference for stepwise mechanism by 4−5 kcal/mol. However, in the case of formamide a competitive situation between both reaction mechanisms has been reported.[42] Therefore, it seems difficult that in the case of undistorted amides, there is a generic reaction mechanism that can be applied to all cases, and the preference for a concerted or a stepwise mechanism depends on the particular amide reactant.

**3.3 Catalytic Effect of the Auxiliary Water Molecule.** The importance of an assiting water molecule in the hydrolysis of amides is well-known and has been emphasized in previous theoretical works for the hydrolysis of formamide.[28,31,32,39] In Tables 1 and 3, we show the effect that the assistant water has

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(55) Krug, J.; Popelier, P.; Bader, R. F. W. *J. Phys. Chem.* **1992**, *96*, 7604−7616.

on the relative free energies by removing this water from the calculations and reoptimizing the corresponding stationary points. The $\Delta\Delta G_{as}$ column in tables 1 and 3 is defined as

$$\Delta\Delta G_{as} = \Delta G - \Delta G_{non-WA} \tag{4}$$

where $\Delta G_{non-WA}$ is the relative free energy in the non water-assisted mechanism. Thus, $\Delta\Delta G_{as}$ is a measure of the catalytic effect of the assistant water molecule, the more negative the $\Delta\Delta G_{as}$, the higher the catalytic effect.

In the case of the twisted amide the order of the catalytic effect for the different transition states is the following one

$$\mathrm{TS1_s}(-10.0) < \mathrm{TS_c}(-12.1) < \mathrm{TS2_s}(-22.2) \tag{5}$$

However in the case of the planar amide **PA** we observe the following order

$$\mathrm{TS_c}(-2.7; -2.2) < \mathrm{TS1_s}(-5.3; -4.9) < \mathrm{TS2_s}(-15.8; -14.3) \tag{6}$$

In both cases, the highest stabilization is obtained for $\mathrm{TS2_s}$, and the lowest stabilization is for $\mathrm{TS_c}$ in **PA** and $\mathrm{TS1_s}$ in **TA**. Overall, **TA** shows a higher reduction in the free energy barriers by the inclusion of the assistant water molecule (more negative $\Delta\Delta G_{as}$) than **PA**, specially in the case of $\mathrm{TS_c}$, for which the value of $\Delta\Delta G_{as}$ is only −2/-3 kcal/mol for **PA** and −12.1 kcal/mol for **TA**. In any case, planar or twisted, the highest stabilizations are obtained for $\mathrm{TS2_s}$, which leads in the case of the twisted amide to a very low barrier for the cleavage of the intermediate.

The origin of the higher sensitivity to an assistant water molecule in the hydrolysis of **TA** is the orthogonality between the nitrogen lone pair ($n_N$) and the carbonyl $\pi$ bond ($\pi_{CO}$). In planar amides, both orbitals are coplanar, with $n_N$ defining the plane of proton transfer and $\pi_{CO}$ the plane of nucleophilic attack, a single water molecule participating in the concerted reaction shows a relatively correct orientation for both proton transfer and nucleophilic attack. Correspondingly, the nonassisted transition state is an almost planar four-member ring (the dihedral defined by $N-C_1-O_{nu}-H_t$ is only 6−8°). In the case of **TA**, and due to the orthogonality between $n_N$ and $\pi_{CO}$, a single water molecule will be less prone to adopt the optimal conformation for the proton transfer and nucleophilic attack. This orientational strain is reflected by a transition state with a high departure from the planarity of the $N-C_1-O_{nu}-H_t$ four-membered ring, the associated dihedral angle being 24°. As a consequence, the transition state of the twisted amide will be favored as compared to the transition state of the planar amide, by the presence of an additional water molecule in the mechanism and the formation of a more flexible six-member ring in the water-assisted mechanism. The additional water molecule will introduce the required extra flexibility to orient optimally the proton for the transfer to $n_N$ and a nucleophilic attack in the $\pi_{CO}$ plane.

***Table 4.*** Relative $\Delta G$'s of the Different Transition States between the Twisted and Planar Amides in kcal/mol for the Structures Optimized in Gas-Phase and in Solution (in bold)

| | $\Delta G_{twist}$ | $\Delta G_{planar}{}^{endo}$ | $\Delta\Delta G^{endo}$ | $\Delta G_{planar}{}^{exo}$ | $\Delta\Delta G^{exo}$ |
|---|---|---|---|---|---|
| $\mathrm{TS_c}$ | 32.7 | 49.3 | 16.6 | 48.3 | 15.6 |
| | **35.5** | **54.5** | **19.0** | **53.6** | **18.1** |
| $\mathrm{TS1_s}$ | 37.2 | 39.8 | 2.6 | 41.6 | 4.4 |
| | **43.8** | **48.0** | **4.2** | **49.8** | **6.0** |
| $\mathrm{TS2_s}$ | 19.8 | 40.7 | 20.9 | 41.9 | 22.1 |
| | **26.4** | **47.8** | **21.4** | **48.5** | **22.1** |

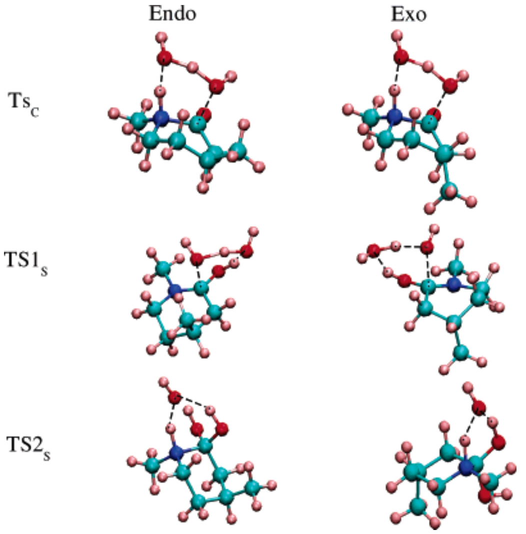

***Figure 4.*** B3LYP/6-31+G(d) structures of the different transition states for the planar amide.

## 4. Discussion

In this section, we compare the difference in energy between the hydrolysis of twisted amide **TA** and planar amide **PA**. To ease the comparison, in Table 4 the energetic barriers of all the transition states are shown. We also give the differential energy barrier, $\Delta\Delta G$, between the planar and twisted amide hydrolysis

$$\Delta\Delta G^{endo} = \Delta G_{planar}{}^{endo} - \Delta G_{twisted} \tag{7}$$

$$\Delta\Delta G^{exo} = \Delta G_{planar}{}^{exo} - \Delta G_{twisted} \tag{8}$$

Thus, a positive $\Delta\Delta G$ indicates a lower barrier for the twisted amide hydrolysis, and therefore, a rate acceleration. We make the comparison between planar and twisted amides taking into account both exo and endo transition states for planar amides. It should be remarked however, that the cage conformation of the twisted amide reactant makes the attack to be necessarily exo with respect to $C_7$.

In general, $\Delta\Delta G$'s are positive indicating that there is a substantial acceleration of the hydrolysis caused by the twisting of the amide bond. However, there is also an interesting dependence of $\Delta\Delta G$'s on the reaction mechanism and the specific transition state considered. The values of $\Delta\Delta G$ ranges from 2.6 to 22.1 kcal/mol, the largest value are found for $\mathrm{TS2_s}$'s, and the lowest ones for $\mathrm{TS1_s}$'s. As a result of these differences, whereas in the case of **PA** (See Figure 5), the two transition states of the stepwise mechanism have similar free energy barriers, and the transition state of the concerted mechanism is higher in energy than $\mathrm{TS1_s}$ or $\mathrm{TS2_s}$, in the hydrolysis of the twisted amide the concerted mechanism is lower in energy, and in the stepwise mechanism, the first step of the reaction is clearly rate-limiting. Thus, our calculations reveal that the twisting of the amide not only has an effect on the relaxation of the barriers in the neutral hydrolysis of amides, but also has a deep mechanistic influence, favoring concerted mechanisms over

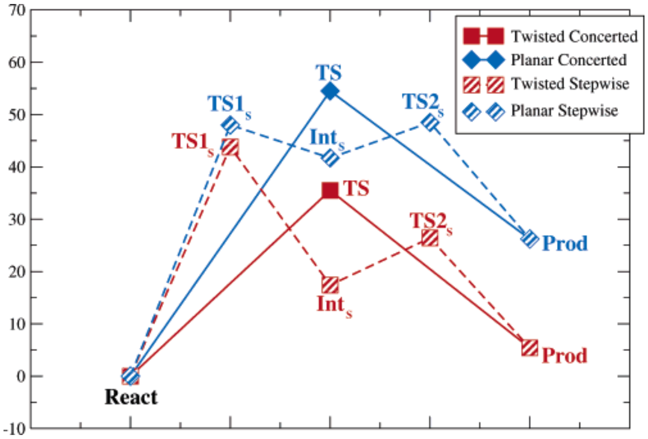

***Figure 5.*** Aqueous free energy reaction profiles (see Methodology) comparing the water-assisted hydrolysis reaction of **TA** and **PA** through a concerted and a stepwise mechanism.

stepwise ones. Consequently, we predict an intriguing relationship between the degree of twist of the amide bond and a predisposition for concerted mechanisms. To understand the trends in $\Delta\Delta G$, one has to bear in mind the electronic characteristics of the amidic unit affected by the twisting of the amide bond. The inherent amidic unit stability has been classically explained by the resonance stabilization model involving a resonance structure in which electronic charge is transferred from the amide nitrogen to the carbonyl oxygen via the $\pi$ system, leading to to a partial double bond character for the C−N bond. This simple model is not without theoretical controversy. According to the work of Wiberg et al.[13−17] charge transfer to oxygen was found to be small upon rotation, and whereas the C−N bond underwent a large change in length on rotation, the C═O bond length changed very little, in contradiction with the resonance model. However, work by Glendening and Hrabal[56] using Natural Population Analysis and Natural Resonance Theory seems to support the traditional resonance model. Besides, Fogarasi et al.[57] pointed out that the analysis of the shifts of Mulliken charges upon rotation instead of Bader's one supported the resonance model, and in addition, the work of Hiberty[58] using valence bond theory also sustained this view. More recently Wiberg et al.[18] have pointed out that the differences can be reconciled if one takes into account the polarity of the C═O bond which will lead to small charge transfer to the oxygen upon rotation. It seems nevertheless that a loss of $n_N \rightarrow \pi_{CO}^*$ delocalization will destabilize the amide and will lead to lower reaction barriers. The barrier heigths of the rotations around the C−N bond[13−18,44] can be taken as a measure of the stability caused by the $n_N \rightarrow \pi_{CO}^*$ delocalization in an undistorted amide, since the rotational transition states show structural characteristics similar to the structure of **TA**, with a nitrogen lone pair orthogonal to the $\pi_{CO}$ orbital plane, an elongated C−N bond and a pyramidalized nitrogen atom. Experimental determination and theoretical calculations on these rotational barriers give values in the order of 15−20 kcal/mol,[59] which are coherent with the calculated barrier relaxation for TS2$_s$ (21.4−22.1 kcal/mol) and TS$_c$ (18.1−19.0 kcal/mol) but not for TS1$_s$ which shows only a moderate $\Delta\Delta G$ of 4−6 kcal/mol. One has to consider, however, that the loss of the $n_N \rightarrow \pi_{CO}^*$ delocalization will also have a sizable effect on the basicities of nitrogen and oxygen. These shifts in basicities with the twist of the C−N bond were recently characterized by estimates of the p$K_a$ values for a series of twisted and planar amides.[7] The calculations revealed that the loss of $n_N \rightarrow \pi_{CO}^*$ delocalization in twisted amides increases the basicity of nitrogen and decreases the one of the oxygen. In fact, highly twisted amides prefer to be protonated at the nitrogen, whereas in planar amides the O-protonated tautomer is more stable, as remarked by Greenberg et al.[6,43] in the context of gas-phase ab initio calculations. The lower basicity of oxygen in twisted amides will unfavor transition states in which the oxygen receives a proton, such as in TS1$_s$, and this unfavorable effect will partially cancel out the barrier relaxation obtained from the ground-state destabilization in the twisted amide reactant.

Whether water-promoted hydrolysis of undistorted amides proceeds through a concerted or stepwise mechanism has stirred considerable debate,[2] in which experimental data input is indirect and scarce. The mechanism for amide hydrolysis in pure water in the absence of acid or base catalysis is difficult to follow by conventional kinetic techniques because they are slow and need to be activated in some way, which obviously affects the comparison.[2] In this respect, theoretical computational studies can be of paramount importance since they can provide with direct data on transition states and their energetic requirements. In this sense, Chalmet et al.[42] found similar barriers for the two steps of the stepwise mechanism in the hydrolysis of formamide. Their conclusion based on a nonassisted mechanism is that both reactions mechanism compete depending on the specific type of amide and conditions involved. Their results for the stepwise mechanism are qualitatively in agreement with our results for **PA**. Isotope effects of the alkaline hydrolysis of formamide[1] support as well the stepwise mechanism with two transition states of similar energy, but it is doubtful how these data can be transferred to the neutral hydrolysis. In summary, it seems that the question of whether the neutral hydrolysis of undistorted amides goes through concerted or stepwise mechanism does not have a clear answer and both mechanism can coexist depending on the specific case involved.

In the case of twisted amides, on the other hand, kinetic studies are difficult because of the fast hydrolysis of the compounds. On the basis of the data presented in this paper, a concerted mechanism is clearly favored (see Figure 5). This is so because of the very high barrier for the first step of the reaction in the stepwise mechanism: the relative free energy of TS1$_s$ is almost 18 kcal/mol higher than the one of TS2$_s$ and 8 kcal/mol higher than the one of TS$_c$. The origin of this high barrier is the requirement of O-protonation in TS1$_s$, which is unfavorable for highly twisted amides, as reported by the work of Greenberg et al.,[6,43] and by our own p$K_a$ estimates in aqueous solvent.[7]

Taking together the results for the hydrolysis of both **TA** and **PA**, and comparing the energetic barriers for the rate-limiting transition states of the lowest-energy mechanism in each case (TS$_c$ for **TA**, and TS1$_s$ for **PA**), we predict a rate-acceleration corresponding to a $\Delta\Delta G$ of 12−13 kcal/mol, sensibly larger than the 7−9 kcal/mol characterized in the case of the alkaline

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hydrolysis of these amides by quantum chemical calculations.[44] This suggests that the degree of rate-acceleration of the hydrolysis of amides upon twisting is highly pH dependent, in line with the experiments of Brown et al.[2,22] who observed a different rate acceleration of twisted amides in basic and acid pH's, a factor of $10^7$ under basic conditions and a factor of a factor of $10^{11}$ under acid-catalyzed conditions. Interestingly, Kirby et al.[23,24] have found that an amide with a similar degree of twist as in **TA** hydrolyzes much faster and suggested that a different mechanism than in the alkaline hydrolysis could be at hand for neutral and slightly acid pH conditions. The calculations presented in this paper are very supportive of a change of the reaction mechanism with the pH and the degree of amide twist. Besides, there are a number of experimental evidences that came along with this finding. Kirby et al. were unable to trap an intermediate in the neutral hydrolysis of a trimethyl variant of 1-Aza-2-adamantanone, an amide with a degree of C−N bond twist similar to the one in **TA**. The intermediate could only be detected at low-pH. Indeed, the comparison of the crystal values for C−N and C−O bond lengths with our theoretical values supports the idea that the hydrolysis intermediate trapped by Kirby et al. corresponds to the acid hydrolysis intermediate (See Results Section). This suggests a fundamental difference in the hydrolytic mechanism as a function of pH, which can be summarized as a propensity for a concerted mechanism at neutral pH, and if the intermediate is formed at acid/alkaline pH will be easily cleaved if the pH is neutralized, due to the low $TS2_s$ barrier.

The findings of the present study of a change in mechanistic pathway with degree of amide bond twist will also be of importance to reveal the mechanism of peptide cleavage processes in biological systems that use ground-state destabilization of the reactant by inducing nonplanarity in the scissile peptide bond. This has been suggested to be at work for example in protein splicing,[8,9] a posttranslational autocatalytic process. It is also considered to be at the basis of the action of some catalytic antibodies, prepared to recognize transition-state analogues of amide hydrolysis reactions. In this sense, our data also provide with relevant geometrical information on the rate-limiting transition states to be reproduced by transition state analogues. We emphasize in this respect, that $TS_c$ and $TS1_s$ show sufficiently different geometrical characteristics (for instance $C_1$−N is 2.348 Å for the former and 1.641 Å for the latter) so that care must be taken as which of the transition state geometries is reflected in a transition state analogue.[10] Our data show that the transition state analogue to be chosen as a hapten in the generation of catalytic antibodies should reproduce the geometrical features of $TS_c$ whenever the binding of the antibody to the reactant induces a significant degree of ground-state destabilization and nonplanarity of the peptide bond.

## 5. Conclusions

In summary, our calculations reveal a tendency for highly twisted amides (with values of $\tau$ close to 90°) to hydrolyze through a concerted mechanism at neutral pH. Additionaly, if the water adduct formes at a different range of pH (acid or alkaline) we predict its fast cleavage whenever the pH is neutralized. Besides, the twist of the C−N bond in the reactant yields the hydrolysis more dependent on the acid/base catalysis provided by an assistant water molecule. All these features combined lead to a radical different picture between the hydrolysis of undistorted amides and twisted ones. This is of considerable interest not only to understand experimental observations in these reactions, but also for a deeper understanding of biological processes in which the cleavage of peptide bond is precursed by ground-state destabilization of the peptide. We also hope that the present theoretical results estimulate further kinetic work on this important set of reactions.

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**Supporting Information Available:** Cartesian coordinates of all the optimized structures (in gas-phase and in solution), full tables containning relative energies enthalpies and free energies for the nonwater-assisted hydrolysis of both amides (twisted **TA** and planar **PA**), and selected geometrical parameters for the key structures (transition states and intermediates) encountered in all reaction mechanisms (water-assisted and nonwater assisted, stepwise and concerted, and **TA** or **PA** as reactant) can be found as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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