Theoretical Study of Amine-Assisted Aminolysis of Penicillins − The Kinetic Role of the Carboxylate Group

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The β-lactam ring opening of 3α-carboxypenam through a methylamine aminolysis reaction catalyzed by another methylamine molecule is studied at the B3LYP/6–31+G* level of theory. Two different neutral mechanisms have been found: a concerted one and a stepwise route through two neutral tetrahedral intermediates. In the gas-phase the most favorable mechanism is stepwise, in which the carboxylate group of 3α-carboxypenam participates directly in the reaction coordinate as a proton shuttle. In aqueous solution the concerted mechanism is the most favored route in which the electrostatic effect of the carboxylate group plays an important role by enhancement of the solute-solvent interaction. The structure and molecular properties of the concerted transition state correlate well with the experimentally reported Brønsted β value and with the greater catalytic advantage of amines compared with water in the aminolysis of penicillins. These effects of the carboxylate group may be of some relevance, not only to understand the aminolysis of β-lactam antibiotics, but also to understand their hydrolysis in aqueous or enzymatic environments.

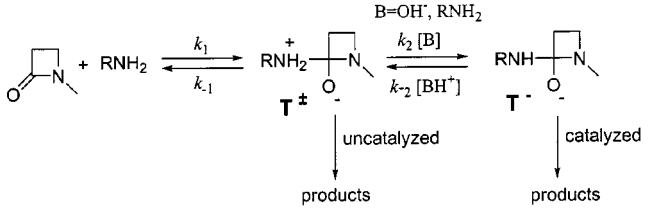
Introduction

The major antigenic determinant of the penicillin allergy detected by the immunological system is the penicilloyl group bound by an amide linkage to the ε-amino groups of lysine residues in plasmid proteins.[1] In addition, allergy skin tests for the determination of IgE antibodies to penicillins consist of the penicilloyl reagent from the aminolysis of the β-lactam ring by polylysine or HSA carrier molecules.[2] To understand further these biochemical processes, the aminolysis of β-lactam compounds has been extensively studied experimentally.[3−6] For a series of primary monoamines, it has been found that the importance of the different reaction pathways for the disappearance of the β-lactam compounds in aqueous amine solution depends on the pK_a, the concentration of the amine, and the pH. The experimentally observed pseudo first-order rate constant k_{obs} is composed of four terms corresponding to different reactive processes. This is expressed by Equation (1):, where k_0 is the first-order rate constant for the hydrolysis reaction and is normally less than 15% of k_{obs}, except at high pH conditions where the alkaline hydrolysis becomes the most important process.

$$k_{obs} = k_0 + k_u[RNH_2] + k_c[RNH_2][RNH_2] + k_{OH}[RNH_2][OH^-] \quad (1)$$

The kinetic constants which correspond to the uncatalyzed (k_u) and the amine-catalyzed (k_c) aminolysis are the predominant terms when weakly basic amines react with β-lactams in the biologically relevant pH range 6−8. For strongly basic amines the amino (k_c) and hydroxide (k_{OH}) catalyzed processes contribute most to the aminolysis reaction — the k_c term is more important as the amine concentration is increased.

Kinetic experiments have provided mechanistic insight into the different routes for aminolysis of β-lactams. Most importantly, the nonlinear dependence of the rate of aminolysis ($k_{obs} - k_0$) of benzylpenicillin and 6-β-aminopenicillanic acid upon hydroxide ion concentration has been interpreted in terms of formation of a *zwitterionic* tetrahedral intermediate $\mathbf{T^{\pm}}$ along a stepwise mechanism (see Scheme 1).[5,6] On the other hand, the Brønsted β-values for the noncatalyzed (k_u) and amine-catalyzed (k_c) aminolysis on the pK_a of amines have values close to unity.[4,5] Following the usual interpretation of the Brønsted plots,[7] it has been assumed that an amine molecule carries a unipositive charge at the corresponding rate-determining Transition Structures (TSs) in the uncatalyzed (k_u) and amine-catalyzed (k_c) routes. This has been considered to be consistent with the stepwise mechanism through *zwitterionic* intermediates $\mathbf{T^{\pm}}$ proposed for both the specific and the general base-catalyzed aminolysis of β-lactams.[5,6]



Scheme 1. Stepwise mechanism via *zwitterionic* intermediates for the specific and general base-catalyzed aminolysis of β-lactams

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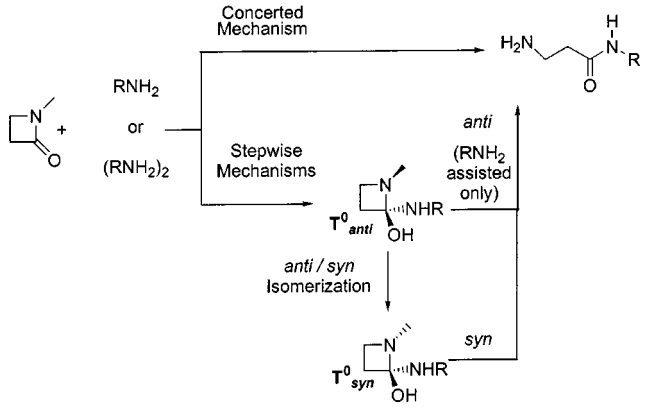
We have performed a series of theoretical studies on the aminolysis of β-lactam compounds in order to obtain a progression of models with detailed descriptions of this interesting process.[8−11] Computational modeling of the ring opening of the simplest β-lactam, 2-azetidinone, by the reaction with either RNH_2 or the RNH_2 dimer (R = H, CH_3),[8−10] has shown that these processes may occur by means of a concerted mechanism or a stepwise pathway through various *neutral* tetrahedral intermediates $\mathbf{T}^0$ (see Scheme 2). The *zwitterions* $\mathbf{T}^{\pm}$ proposed in Scheme 1 were theoretically optimized in solution and found to be very unstable intermediate species with a very short mean lifetime before fragmentation into reactants, even in strongly polar media.[10] Therefore the formation of an encounter complex between a *zwitterionic* intermediate and a basic catalyst, and its subsequent rupture to products, would be a very unlikely event. The most favorable mechanism for both the purely uncatalyzed (k_u) and the amine-assisted (k_c) reactions of *monocyclic* β-lactams begin with nucleophilic attack of RNH_2 on the carbonyl group and lead to a neutral tetrahedral intermediate[12] $\mathbf{T}^0_{anti}$, in which the attacking group and the *N*-substituent have an antiperiplanar relationship which maximizes charge transfer from nucleophile to β-lactam. The most favorable evolution of $\mathbf{T}^0_{anti}$ proceeds through a $\mathbf{T}^0_{anti} \rightarrow \mathbf{T}^0_{syn}$ isomerization followed by a hydrogen transfer between the hydroxy and the forming amino group with simultaneous cleavage of the β-lactam ring (see Scheme 2). The catalytic moiety resembles an RNH_3^+ cation at the main transition states (TSs) for the amine-assisted reaction of 2-azetidinone. This is consistent with the experimental interpretation of the Brønsted plots for k_c for monocyclic and bicyclic β-lactams.[4,5] Moreover, the calculated activation Gibbs energies in aqueous solution for these processes (around 40 kcal mol^{-1} with respect to separate reactants) are also consistent with the experimentally observed slow kinetics for the reaction of benzylpenicillin with monoamines.[5]

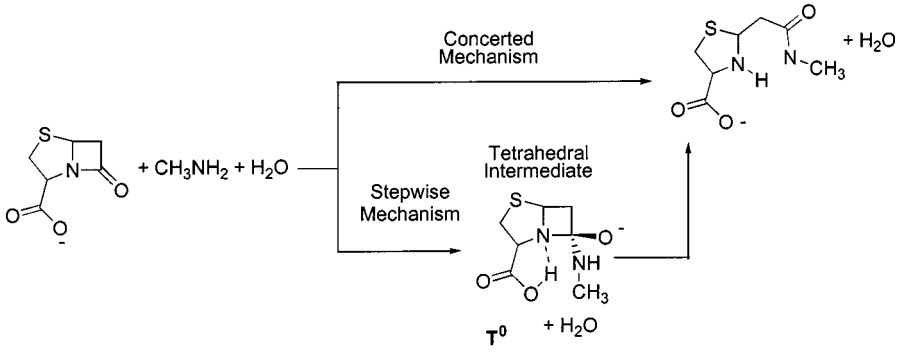


Scheme 2. Theoretically proposed mechanisms for uncatalyzed and amine-catalyzed aminolysis reaction of 2-azetidinone

Very recently, the specific role of solvent molecules in the mechanism of the aminolysis of monocyclic β-lactams has been studied theoretically.[11] In this case, the most favorable mechanism is stepwise with an *anti*/*syn* isomerization similar to that sketched in Scheme 2. The catalytic action of one water molecule is around 13 and 9 kcal mol^{-1} in Gibbs energy in the gas phase and in solution, respectively. These theoretical data indicate that a purely uncatalyzed mechanism for the aminolysis reaction of monocyclic β-lactams in aqueous solution is not competitive with the water-assisted mechanism which therefore should be the most important contribution to the k_u term.

For bicyclic systems like 3α-carboxypenam, in which the *anti*/*syn* isomerization is impeded, theoretical calculations[11] have revealed new mechanistic aspects owing to the presence of the thiazolidine ring (see Scheme 3). The most favored mechanism for water-assisted aminolysis of 3α-carboxypenam in aqueous solution is predicted to be the concerted one, which is in contrast with earlier proposals.[5,6] Nevertheless, the presence of a positive charge on the nucleophilic moiety in the concerted transition structure (absent in the stepwise rate-determining TS) explains well the reported positive Brønsted β value for the uncatalyzed reaction of benzylpenicillin with a series of amines. It has also been observed that the carboxylate group of penicillins can exert a large kinetic influence either as a proton shuttle in the stepwise mechanism[13] (see $\mathbf{T}^0$ in Scheme 3) or by enhancement of the solute-solvent interactions along the concerted reaction profile. Therefore the kinetic role of the carboxylate group outlined in our previous work could have some interest, not only to understand the water-assisted aminolysis of β-lactam antibiotics, but also for other processes in the chemistry of β-lactams.[14]

In this work we report on a theoretical investigation of the methylamine-assisted aminolysis of the penicillin model



Scheme 3. Theoretically proposed mechanisms for the H_2O-assisted aminolysis of the 3α-carboxypenam

compound 3α-carboxypenam which complements our theoretical studies on the β-lactam models through the analysis of the k_c term in Equation (1). The ability of the theoretical neutral mechanisms to rationalize the experimental data on the aminolysis of β-lactams will be further discussed, and the catalytic action of water and methylamine will be compared. The theoretical consideration of this penicillin model may allow us to predict more clearly the concerted/stepwise kinetic preference for the aminolysis of β-lactam antibiotics. In addition, the kinetic effect of the carboxylate group present in the 3α-carboxypenam model compound will also be characterized.

Computational Methods

Molecular geometry optimizations followed by analytical frequency calculations were performed at the HF/3−21G* and B3LYP/6−31+G* levels of theory[15,16] using the Gaussian 98[17] suite of programs. Although the preliminary HF/3−21G* results are not presented in this work, Intrinsic Reaction Coordinate (IRC) calculations[18] at the HF/3−21G* level were carried out to confirm the reaction paths on the Potential Energy Surface (PES) which connect the most important transition structures, intermediates and products. $\Delta G_{\text{gas-phase}}$ values were obtained by combination of the B3LYP/6−31+G* electronic energies and thermal corrections[19] at the same level of theory. In previous work,[11] the ability of the B3LYP/6−31+G* method to study the aminolysis of the 3α-carboxypenam anion was tested by a favorable comparison between the B3LYP/6−31+G* results on the ammonolysis of the model compound 2-formamide-acetate and the G2(MP2,SVP) results.

The condensed-phase effects on the kinetics and thermodynamics of the reaction were taken into account by use of the recently derived UAHF (united atom Hartree−Fock) parameterization of the polarizable continuum model (PCM)[20] with water simulated as solvent. The solvation Gibbs energies $\Delta G_{\text{solvation}}$ of all the critical structures were then computed from single-point B3LYP/6−31+G* PCM-UAHF calculations on the B3LYP/6−31+G* gas-phase geometries. Addition of the relative solvation Gibbs energies to the $\Delta G_{\text{gas-phase}}$ values gives the $\Delta G_{\text{solution}}$ values in Table 1.

Atomic charges were computed with a Natural Population Analysis (NPA) using the corresponding B3LYP/6−31+G* density matrices.[21]

Results and Discussion

The exploration of the PES for the methylamine-assisted reaction between 3α-carboxypenam and methylamine gave a concerted and a stepwise mechanisms, which are comparable to those found for the water-assisted reaction[11] (see Scheme 3). The optimized geometries of the structures along both reaction paths are shown in Figure 1. Table 1 collects the relative energies along the reaction profiles including the ZPVE correction from the B3LYP/6−31+G* unscaled frequencies, and the corresponding relative Gibbs energy values. Figure 2 and 3 show the Gibbs energy profiles in the gas-phase and in solution, respectively.

The concerted and stepwise mechanisms start with the same prereactive complex **C** in which the methylamine dimer interacts with one of the carboxylate oxygen atoms of 3α-carboxypenam through a strong H-bond.[22] Complex **C** is stabilized by 12.7 kcal mol^{-1} with respect to the separate reactants. When thermal corrections are taken into account, however, **C** becomes a transient structure along the reaction coordinate, 5.6 kcal mol^{-1} above the reactants in gas-phase Gibbs energy.

Concerted Mechanism

The nucleophilic attack of CH_3NH_2 on the less-hindered face (α-face) of the carbonyl group in 3α-carboxypenam leads to a TS for the concerted aminolysis (see $\mathbf{TS_C}$ in Figure 1). $\mathbf{TS_C}$ presents a tight structure with a single C−N bond almost formed (1.555 Å), and an endocyclic C−N bond partially cleaved (1.642 Å). A hydrogen atom has been transferred from the nucleophilic amine to the catalyst, while the catalytic amine has not yet transferred a proton to the endocyclic nitrogen atom. Therefore, $\mathbf{TS_C}$ is best described as a $CH_3NH_3^+$ fragment (with an NPA charge of

Table 1. Relative energies and Gibbs energies in the gas-phase and in aqueous solution, and solvation energies (kcal mol^{-1}) with respect to reactants of the structures considered in the CH_3NH_2-assisted aminolysis reaction of 3α-carboxypenam; values in parentheses correspond to the water-assisted aminolysis reaction (see ref.[11])

Structures	B3LYP/6−31+G*[a]	$\Delta G_{\text{gas-phase}}$	$\Delta\Delta G_{\text{solvation}}$	$\Delta G_{\text{solution}}$
Reactants	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
C	−12.7(−18.5)	5.6(−0.8)	21.3(27.8)	26.9(27.0)
TS_C	25.1 (22.9)	49.5 (45.0)	−5.1 (9.4)	44.4(54.4)
TS_1	14.6 (8.5)	38.5 (31.0)	11.2(19.8)	49.7 (50.7)
I_1	11.6 (6.8)	34.1(28.7)	12.4(12.3)	46.5(41.1)
$TS_{I1\rightarrow I2}$[b]	12.0 (−)	36.2 (−)	18.3 (−)	54.5 (−)
I_2	10.0 (3.4)	31.2 (24.2)	18.9(22.7)	50.1(46.9)
TS_2	10.1 (4.2)	32.4 (25.1)	32.0(33.2)	64.4(58.3)
P	−38.3 (−43.8)	−18.6 (−24.2)	19.4(21.2)	0.8(−3.0)
Products	−31.7	−20.9	7.7	−13.2

[a] Including ZPVE correction. − [b] Structure not located for the water-assisted mechanism due to the extremely flat character of the B3LYP/6-31+G* PES.

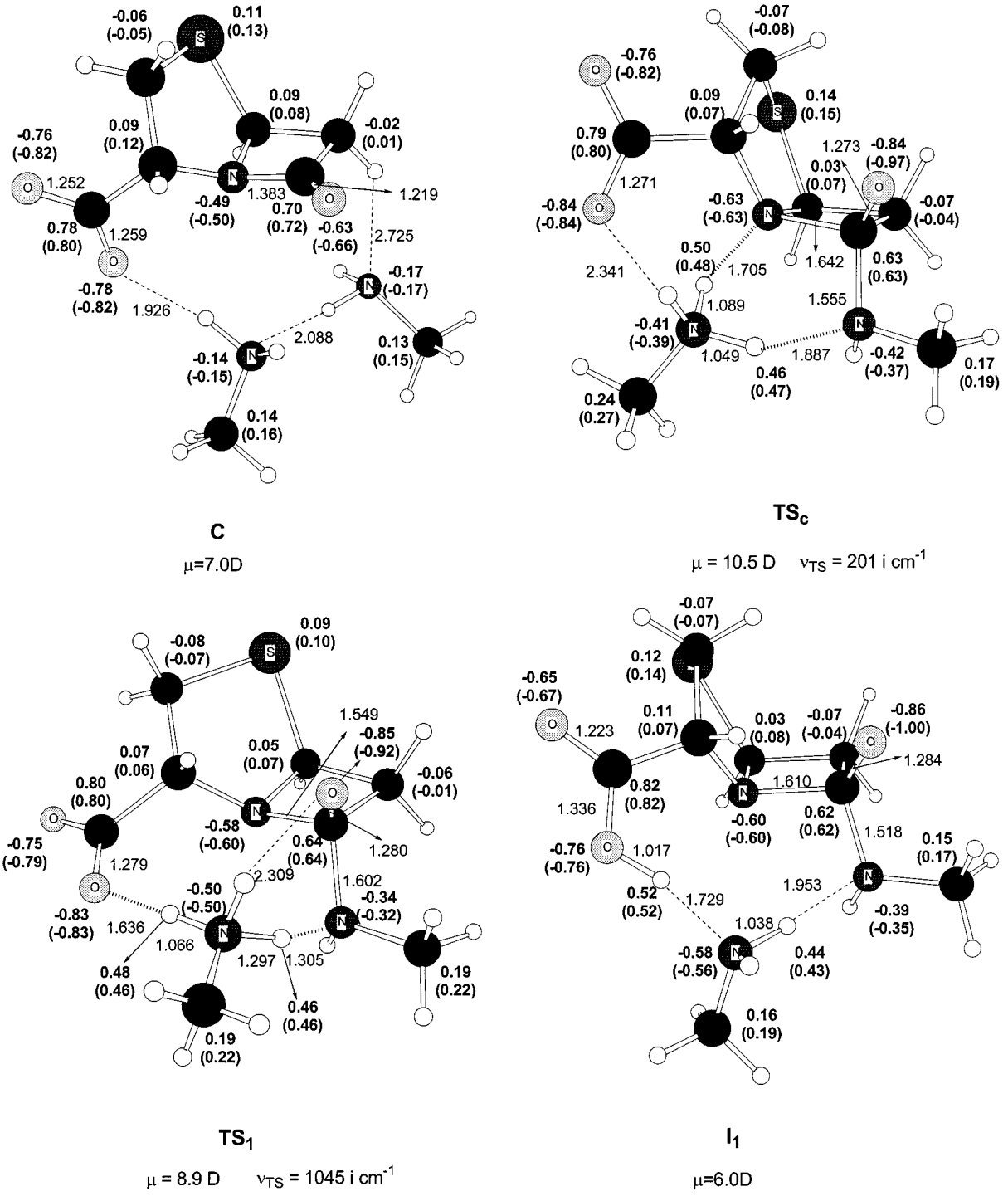


Figure 1. B3LYP/6−31+G* optimized structures for the CH_3NH_2-assisted reaction between methylamine and 3α-caboxypenam; distances in Angstroms; gas-phase dipole moments (in Debyes), atomic charges (in e, boldface) from Natural Population Analysis with hydrogens summed into the heavy atoms (values in solution within parentheses), and the frequency corresponding to the transition vector (in cm^{-1}) are also displayed

+0.79 e) which interacts with a methylamino-alcoholate dianion (see Figure 1). The $\Delta G_{gas\text{-}phase}$ barrier of **TS$_C$** is 49.5 kcal mol^{-1}.

From **TS$_C$** the transfer of a proton from the $CH_3NH_3^+$ catalyst to the endocyclic nitrogen atom and the opening of the β-lactam ring yields the product complex **P**. In **P**, which has a $\Delta G_{gas\text{-}phase}$ value of −18.6 kcal mol^{-1}, the catalytic methylamine interacts with the penicilloyl moiety through two H-bonds with N···HN and NH···O distances of 2.872 and 2.007 Å, respectively. The $\Delta G_{gas\text{-}phase}$ binding energy

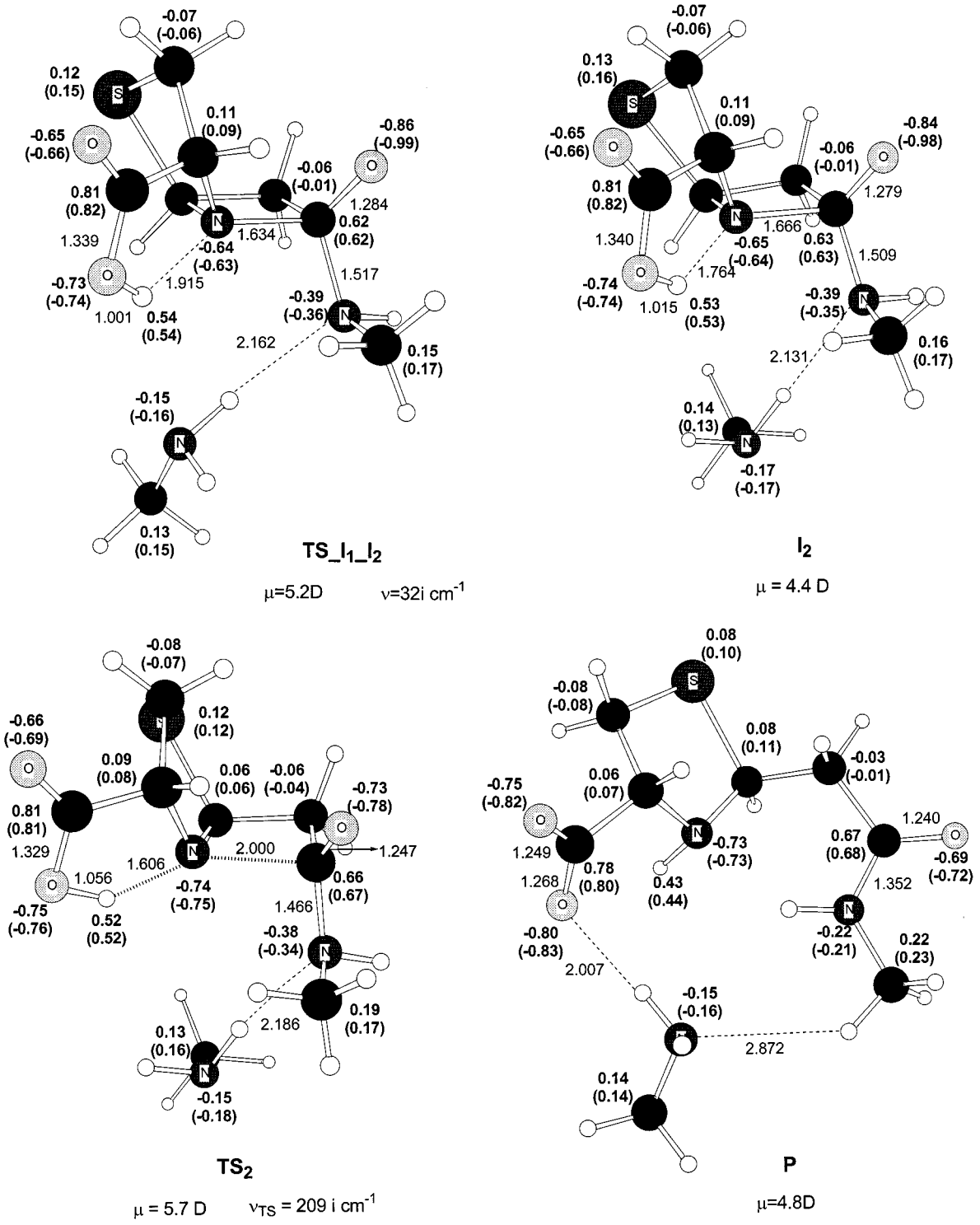


Figure 1. *(Continued)*

between the ancillary amine molecule and the penicilloyl group from 3α-carboxypenam and methylamine amounts to 2.3 kcal mol^{-1}, the ΔG_{rxn} term in the gas-phase is thus −20.9 kcal/mol.

Stepwise Mechanism

We have previously characterized[10] a stepwise mechanism for the CH_3NH_2-assisted aminolysis of 2-azetidinone.

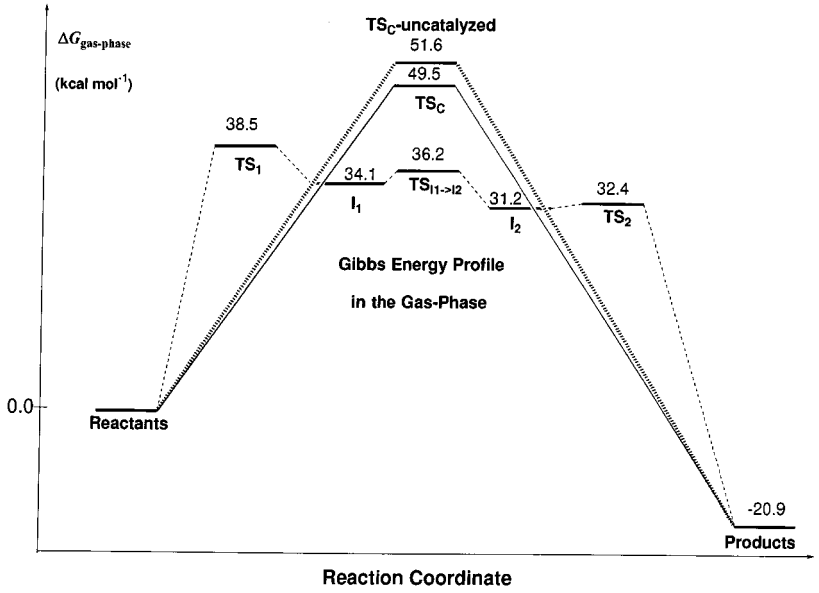


Figure 2. Gibbs energy profiles (kcal/mol) in the gas-phase for the different mechanisms studied in this work

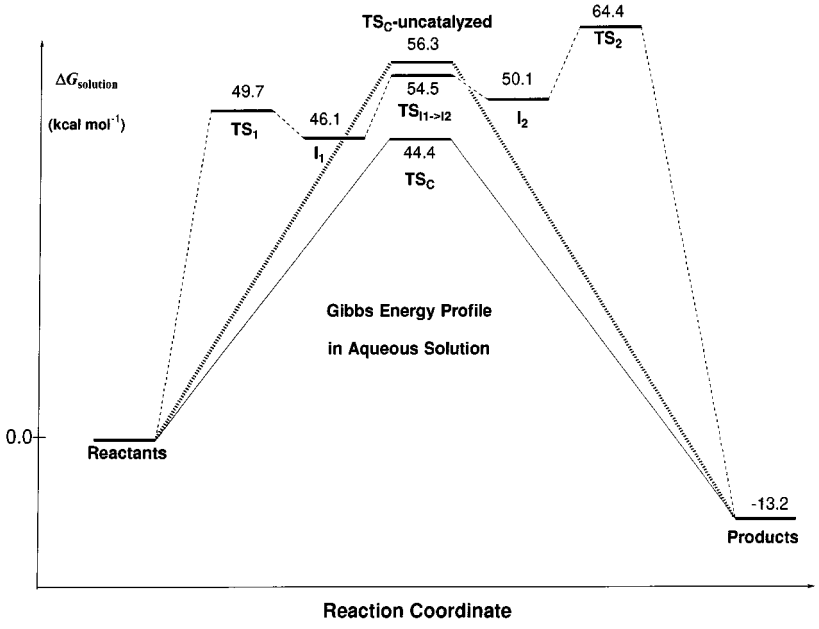
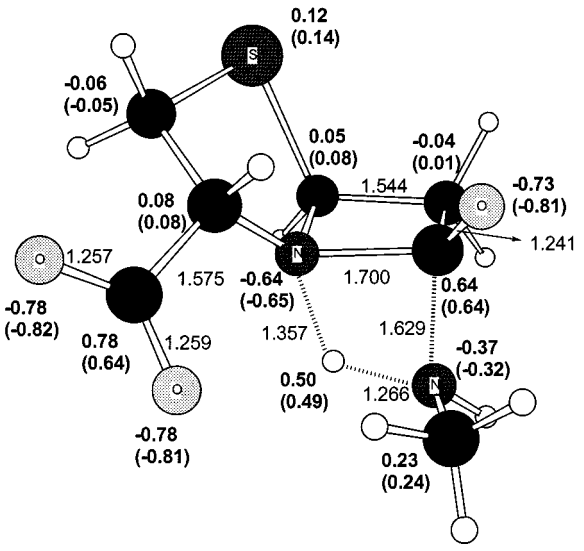


Figure 3. Gibbs energy profiles (kcal/mol) in solution for the different mechanisms studied in this work

The first step consists of the nucleophilic attack on the carbonyl group with simultaneous CH_3NH_2-mediated proton transfer from the nucleophile to the carbonylic oxygen. In the case of the stepwise aminolysis of 3α-carboxypenam, intensive searches on the PES gave **TS$_1$**. In this case, most interestingly, the initial attack of the amine implies an amine-assisted H-transfer to a carboxylic O atom instead of the carbonylic O atom (see Figure 1). In **TS$_1$**, the active H-atoms at the catalytic moiety establish short H-bonds[22] bridging the nucleophile and the carboxylic group with equilibrium NH···N and O···HN distances of 1.305 and 1.636 Å, respectively. Consequently, **TS$_1$** clearly presents a less ionic character than **TS$_C$** (see dipole moments in Figure 1), and **TS$_1$** has a $\Delta G_{gas\text{-}phase}$ value of 38.5 kcal mol^{-1}, 11.0 kcal mol^{-1} below **TS$_C$**.

TS$_1$ is connected to an amino-alcoholate, intermediate **I$_1$**, which has a $\Delta G_{gas\text{-}phase}$ value of 34.1 kcal mol^{-1}, 4.4 kcal mol^{-1} below **TS$_1$**. In this intermediate, the catalytic methylamine bridges the amino-alcoholate moiety and the carboxylate group through two H-bonds of 1.729 Å and 1.953 Å. Intermediate **I$_1$** evolves to a second intermediate **I$_2$** through a TS which reorientates the hydrogen atom transferred to the carboxy group (**TS$_{I1\rightarrow I2}$** in Figure 1) so that it is ready to interact with the lone pair of the endocyclic nitrogen atom. **I$_2$** has a $\Delta G_{gas\text{-}phase}$ value of 31.2 kcal mol^{-1}. In **I$_2$** the catalytic methylamine fragment is most favorably linked by a single H-bond to the exocyclic amino group while the neutral carboxylic group and the fully pyramidalized endocyclic N-atom establish a short H-bond with an OH···N distance of 1.764 Å. It is worthwhile to



TS_C-uncatalyzed

μ = 9.5 D $\quad \nu_{TS}$ = 1579 i cm^{-1}

Figure 4. B3LYP/6−31+G* optimized TS for the uncatalyzed reaction between methylamine and 3α-caboxypenam; distances in Angstroms; gas-phase dipole moments (in Debyes), atomic charges (in e, boldface) from Natural Population Analysis with hydrogens summed into the heavy atoms (values in solution within parentheses), and the frequency corresponding to the transition vector (in cm^{-1}) are also displayed

note that unlike the $\mathbf{T}^{\pm}$ species proposed in Scheme 1, both intermediates $\mathbf{I_1}$ and $\mathbf{I_2}$ have no *zwitterionic* character.

Clearly, the intramolecular OH···N contact at $\mathbf{I_2}$ is suitable for the transfer of the H-atom from the carboxylic group to the amino group, thus facilitating the ring opening of the β-lactam. In fact $\mathbf{I_2}$ may evolve into the product **P** through a TS ($\mathbf{TS_2}$ in Figure 1) in which the endocyclic C−N bond is substantially broken whereas the simultaneous H-shift from the carboxylic group to the forming amino group remains at its initial stage. $\mathbf{TS_2}$ has a $\Delta G_{\text{gas-phase}}$ value of 32.4 kcal mol^{-1}, only 1.2 kcal mol^{-1} above $\mathbf{I_2}$ and 6.1 kcal mol^{-1} below $\mathbf{TS_1}$.

Therefore, our calculations predict that the most favorable route for the aminolysis of the bicyclic system 3α-carboxypenam in the gas-phase is the stepwise one, with $\mathbf{TS_1}$, for the formation of the intermediate $\mathbf{I_1}$, as the rate determining TS (see Figure 2). Interestingly, in this stepwise mechanism the H-transfer to the forming amino group proceeds through both the catalytic CH_3NH_2 molecule and the carboxylate group.

Solvent Effects

In view of the nature of the mechanisms found in the gas-phase, the solvent is expected to have a considerable impact on this process. To estimate the solvent effect the relative solvation energies with respect to reactants ($\Delta\Delta G_{\text{solvation}}$) were evaluated by means of PCM calculations in the gas-phase geometries. Although full optimization of the critical structures in solution was impeded by numerical difficulties, we expect that the main features of the PES in solution can be reasonably outlined from our calculations.

From the $\Delta\Delta G_{\text{solvation}}$ values in Table 1, it is evident that the solvent can have a decisive kinetic influence on the process (see Figure 2 and 3). Thus, in the $\mathbf{TS_C}$ the negative charge on the carboxylate group and on the carbonylic O atom, and the positive charge on the catalytic amine (see above) produce a strong polarization of the continuum. As a consequence, $\mathbf{TS_C}$ becomes the most stable TS in solution with a $\Delta G_{\text{solution}}$ barrier of 44.4 kcal mol^{-1}. Along the stepwise mechanism, the more advanced a structure, the lower the charge separation, and consequently the destabilization by the solvent is greater. Addition of the $\Delta\Delta G_{\text{solvation}}$ terms to the $\Delta G_{\text{gas-phase}}$ renders the intermediate $\mathbf{I_1}$ 3.2 kcal mol^{-1} below its precursor $\mathbf{TS_1}$. Solvent effects destabilize $\mathbf{I_2}$ with respect to $\mathbf{I_1}$ by 3.6 kcal mol^{-1}. Since $\mathbf{TS_2}$ for the stepwise ring-opening of the β-lactam is the TS with the least-polar character, inclusion of the solvation energies predicts that $\mathbf{TS_2}$ would be the rate-determining step with a high Gibbs energy barrier of 64.4 kcal mol^{-1}, 14.7 and 20.0 kcal mol^{-1} above those of $\mathbf{TS_1}$ and $\mathbf{TS_C}$, respectively. These clear energetic differences lead us to predict that, in aqueous solution, the concerted mechanism would become the most favored. The solvent diminishes the thermodynamic driving force of the process since the calculated ΔG_{rxn} of this process in solution (0.8 kcal mol^{-1}) is 19.4 kcal mol^{-1} in absolute value below the gas-phase value.

Uncatalyzed Mechanism

For comparative purposes, we also used B3LYP/6−31+G* to study the uncatalyzed reaction between 3α-carboxypenam and methylamine through a concerted mechanism (see **TS_C-uncatalyzed** in Figure 4). This TS corresponds to the 1,2-addition of one N−H bond of the attacking amine to the C−N amide bond. As in the case of $\mathbf{TS_C}$, the new C−N bond (1.629 Å) is practically formed at **TS_C-uncatalyzed** whereas the β-lactam ring is barely opened (1.700 Å). In the gas-phase, the barrier for this mechanism is 14.2 kcal mol^{-1} (B3LYP/6−31+G* energies) and 2.0 kcal mol^{-1} ($\Delta G_{\text{gas-phase}}$) above that of the methylamine-assisted $\mathbf{TS_C}$. With respect to the water-assisted mechanism, the uncatalyzed process is disfavored by 16.4 kcal mol^{-1} (B3LYP/6−31+G* energies) and 6.5 kcal mol^{-1} ($\Delta G_{\text{gas-phase}}$). We see in Figure 3 that the inclusion of solvent effects increases the Gibbs energy difference between the concerted amine-assisted process and the purely uncatalyzed route, the catalytic effect of the second amine molecule is thus 11.9 kcal mol^{-1} in terms of $\Delta G_{\text{solution}}$ values. However, the stepwise route would also be disfavored in solution with respect to the uncatalyzed pathway whose $\Delta G_{\text{solution}}$ barrier (56.3 kcal/mol) is 8.2 kcal mol^{-1} below that of $\mathbf{TS_2}$ (see Figure 3).

Comparison Between the Water-Assisted and Amine-Assisted Gibbs Energy Profiles

It is interesting to perform a comparative analysis of the catalytic effect of methylamine and water in the aminolysis of 3α-carboxypenam. The catalytic actions of water and

methylamine are quite different. For both $\mathbf{TS_C}$ and $\mathbf{TS_1}$, the catalytic water molecule resembles a hydroxide ion moiety whereas the catalyst has a methylammonium character in the amine-assisted structures. In the gas-phase the ΔG profile for the water-assisted process is about 4−8 kcal mol^{-1} more stable relative to the reactants than that for the methylamine-assisted reaction, and the energy barrier which corresponds to $\mathbf{TS_1}$ is 7.5 kcal mol^{-1} lower than when water acts as a catalyst. In solution, the catalytic action of water is still more efficient than that of methylamine in the stepwise mechanism. On the contrary, in the concerted mechanism, which is the most favorable one in solution, the most efficient catalyst is methylamine, by 10 kcal mol^{-1}.

Comparison with Experiment

For the reaction of monoamines with benzylpenicillin and other β-lactams in aqueous solutions, the structure of the rate-determining TS has been experimentally characterized from the linear relationship between the rate constants k_u or k_c and the pK_a of the conjugate acid of the base [Equation (2)].[7]

$$\log k = A + \beta\, \mathrm{p}K_a \quad (2)$$

Here, A is a constant for the particular reaction while the Brønsted β-value measures the sensitivity of the reaction to the basic strength of the amine molecule. For base catalysis, the values of β are always between 0 and 1: complete proton transfer to the catalyst at the rate-determining TS results in a β value of 1 and no transfer a value of 0. Thus, β can be interpreted as a measure of the charge of the catalyst at the transition state. We note that when there is acid-base catalysis partly neutralizing the charges formed at the transition state there is no direct relation between β and the extent of bond formation.[23]

$\Delta G_{solution}$ values in Table 1 clearly predict a concerted mechanism as the most favored for the aminolysis reaction of penicillinic antibiotics catalyzed by amines in aqueous solution. This theoretical prediction is in agreement with the experimentally[5] found Brønsted β value of 1.0 thanks to the unequivocal presence of the protonated form of the methylamine catalyst in $\mathbf{TS_C}$ (see Figure 1). As previously mentioned, the geometry of the catalytic methylamine moiety at $\mathbf{TS_C}$ supports a formal partitioning of $\mathbf{TS_C}$ into two interacting ionic moieties ($CH_3NH_3^+$···amino-alcoholate dianion). The partitioning of the charge density into atomic contributions by means of the Natural Population Analysis is also consistent with the β value of 1.0, the methylamine fragment having a positive charge of +0.79 e in the gas-phase and +0.83 e in aqueous solution.

Previous work on the amine-assisted aminolysis of monocyclic β-lactams has shown that the Brønsted β values close to unity are compatible with both the concerted and nonconcerted routes, the positive charge is located on the conjugate-acid of the catalytic amine molecule. This contrasts sharply with the case of the aminolysis of the bicyclic system 3α-carboxypenam. In this case, the TS for the concerted mechanism is compatible with the Brønsted β values, whereas the structure, transition vector, and charge distribution of $\mathbf{TS_2}$ (see above), which describe the rate-determining process in solution in the stepwise pathway, cannot explain the experimentally observed dependence of the rate constant on the pK_a of the catalytic amine.

If the so-called uncatalyzed process is interpreted as catalyzed by water in agreement with theoretical studies,[11] the experimentally reported kinetic constants k_c and k_u for propylamine and butylamine show a difference in activation energy of about 5 kcal mol^{-1} in favor of the amine-catalyzed process.[5a] Our calculations qualitatively predict this experimental fact with the concerted route as the most favored given that in the stepwise mechanism the catalytic action of water is more effective than that of methylamine.

Conclusions

Quantum chemical calculations clearly predict a concerted mechanism as the most favored for the aminolysis reaction of penicillins catalyzed by amines in aqueous solution. The structure and molecular properties of the concerted transition state correlate well with the experimentally determined Brønsted β value of 1.0, and the greater catalytic advantage of methylamine compared with water. This kinetic preference for the concerted route and the absence of the *zwitterionic* tetrahedral intermediates are in contrast with earlier mechanistic proposals. The ability of the theoretical neutral mechanisms to explain the aminolysis of β-lactams gains further support in this study.

Our results have revealed a notable kinetic influence of the penicillin carboxylate group. On the one hand, this group may act directly as a proton shuttle in the stepwise mechanism, the most favored in the gas-phase. On the other hand, its electrostatic effect enhances the solute-solvent interactions along the concerted-reaction profile which in turn reverses the concerted/stepwise mechanistic preference in contrast with the 2-azetidinone case. These effects may be of some relevance, not only to understand the aminolysis of β-lactam antibiotics, but also to understand the hydrolysis of β-lactams in aqueous or enzymatic environments.

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[1] [1a] B. B. Levine, Z. Ovary, *J. Exp. Medicine* **1961**, *114*, 875−904. − [1b] C. W. Parker, J. Shapiro, M. Ken, H. N. Eisen, *J. Exp. Medicine* **1962**, *115*, 821. − [1c] A. L. de Weck, *Int. Arch. Allergy Appl. Immunol.* **1962**, *21*, 20. − [1d] M. Blanca, J. M. Vega, J. García, A. Miranda, M. J. Carmona, C. Juárez, S. Terrados, J. Fernández, *Clinical and Experimental Allergy* **1994**, *24*, 407−415

[2] A. Saxon, G. N. Beall, A. S. Rohr, D. C. Adelman, *Ann. Intern. Med.* **1987**, *107*, 204−215.

[3] T. Yamana, A. Tsuji, E. Miyamoto, E. Mira, *J. Pharm. Pharmac.* **1975**, *27*, 56−57.

[4] G. M. Blackburn, J. D. Plackett, *J. Chem. Soc., Perkin Trans. 2* **1973**, 981−985.

[5] [5a] J. J. Morris, M. I. Page, *J. Chem. Soc., Perkin Trans. 2* **1980**, 212−219. − [5b] J. J. Morris, M. I. Page, *J. Chem. Soc., Perkin Trans. 2* **1980**, 220−224.

[6] [6a] M. I. Page, *Acc. Chem. Res.* **1984**, *17*, 144. – [6b] M. I. Page, *The mechanism of Reactions of β-lactams*, in *The Chemistry of β-lactams* (Ed.: M. I. Page) Blackie Academic & Professional, London, **1992**, pp. 129–147.
[7] C. D. Ritchie, *Physical Organic Chemistry*, Marcel Dekker Inc., **1990**, New York.
[8] N. Díaz, D. Suárez, T. L. Sordo, *Chem. Eur. J.* **1999**, *5*, 1045–1054.
[9] N. Díaz, D. Suárez, T. L. Sordo, *J. Org. Chem.* **1999**, *64*, 3281–3289.
[10] N. Díaz, D. Suárez, T. L. Sordo, *J. Org. Chem.* **1999**, *64*, 9144–9152.
[11] N. Díaz, D. Suárez, T. L. Sordo, *J. Am. Chem. Soc.* **2000**, *122*, 6710–6719
[12] Interestingly, the participation of neutral tetrahedral intermediates T0 in the alcoholysis reaction of cephalosporins has been proposed in order to explain the experimental kinetics in the pH range 6–8. See K. J. Davies, M. I. Page, *J. Chem. Soc., Chem. Commun*. **1990**, 1448–1450.
[13] A similar mechanism has been described by Wolfe for the water-assisted methanolysis of 3-carboxypenam at the AM1 level. See: S. Wolfe, *Can. J. Chem.* **1994**, *72*, 1014–1032.
[14] D. J. Tantillo, J. Chen, K. N. Houk, *Curr. Opin. Chem. Biol.* **1998**, *2*, 743–750, and references therein.
[15] W. J. Hehre, L. Radom, J. A. Pople, P. v. R. Schleyer, *Ab initio Molecular Orbital Theory*, John Wiley & Sons Inc., New York, **1986**.
[16] A. D. Becke, in *Modern Electronic Structure Theory, Part II* (D. R. Yarkony, Ed.), World Scientific, Singapore **1995**, 1022.
[17] Gaussian 98, Revision A.6: M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh, **1998**.
[18] [18a] K. Fukui, *Acc. Chem. Res.* **1981**, *14*, 363. – [18b] C. González, H. B. Schlegel, *J. Phys. Chem.* **1990**, *94*, 5523.
[19] D. A. McQuarrie, *Statistical Mechanics*, Harper & Row, New York, **1976**.
[20] [20a] J. Tomasi, M. Persico, *Chem. Rev.* **1994**, *94*, 2027–2094. – [20b] J. Tomasi, R. Cammi, *J. Comput. Chem.* **1996**, *16*, 1449–1458. – [20c] V. Barone, M. Cossi, J. Tomasi, *J. Chem. Phys.* **1997**, *107*, 3210–3221.
[21] A. E. Reed, R. B. Weinstock, F. Weinhold, *J. Chem. Phys.* **1985**, *83*, 735
[22] J. A. Gerlt, M. M. Kreevoy, W. W. Cleland, P. A. Frey, *Chem. Biol.* **1997**, *4*, 259–267.
[23] A. R. Fersht, *J. Am. Chem. Soc.* **1971**, *93*, 3504.

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