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Computational Study on Mechanistic Details of the Aminoethanol Rearrangement Catalyzed by the Vitamin B_{12}-Dependent Ethanolamine Ammonia Lyase: His and Asp/Glu Acting Simultaneously as Catalytic Auxiliaries

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The rearrangement of aminoethanol catalyzed by ethanolamine ammonia lyase is investigated by computational means employing DFT (B3LYP/6-31G*) and ab initio molecular orbital theory (QCISD/cc-pVDZ). The study aims at providing a detailed account on various crucial aspects, in particular a distinction between a direct intramolecular migration of the partially protonated NH_2 group vs elimination of NH_4^+. Three mechanistic scenarios were explored: (i) According to the calculations, irrespective of the nature of the protonating species, intramolecular migration of the NH_3 group is energetically less demanding than elimination of NH_4^+. However, all computed activation enthalpies exceed the experimentally derived activation enthalpy (15 kcal/mol) associated with the rate-determining step, i.e., the hydrogen abstraction from the 5′-deoxyadenosine by the product radical. For example, when imidazole is used as a model system for His interacting with the NH_3 group of the substrate, the activation enthalpy for the migration process amounts to 27.4 kcal/mol. If acetic acid is employed to mimic Asp or Glu, the activation enthalpy is somewhat lower, being equal to 24.2 kcal/mol. (ii) For a partial deprotonation of the substrate **2** at the OH group, the rearrangement mechanism consists of the dissociation of an NH_2 radical from C(2) and its association at C(1) atom. For all investigated proton acceptors (i.e., OH^-, $HCOO^-$, CH_3COO^-, CH_2-NH, imidazole), the activation enthalpy for the dissociation step also exceeds 15 kcal/mol. Typical data are 20.2 kcal/mol for Ac^- and 23.8 kcal/mol for imidazole. (iii) However, in a synergistic action of partial protonation of the NH_2 group and partial deprotonation of the OH group by the two conceivable catalytic auxiliaries Asp/Glu and His, the activation enthalpy computed is compatible with the experimental data. For imidazole and acetate as model systems, the activation enthalpy is equal to 13.7 kcal/mol. This synergistic action of the two catalytic groups is expected to take place in a physiologically realistic pH range of 6−9.5, and the present computational findings may help to further characterize the yet unknown structural details of the ethanolamine ammonia lyase's active site.

Introduction

Ethanolamine ammonia lyase[1] is a bacterial enzyme which metabolizes the substrate aminoethanol,[2] **1**, to ethanal, **11**, and ammonia (Scheme 1).[3] The enzyme requires the presence of the B_{12} coenzyme for its catalytic activity. In the first step of the reaction, the homolytic cleavage of the C−Co(III) bond in the vitamin B_{12} coenzyme is assumed to generate the low-spin Cob(II)-alamin and a 5′-deoxyadenosyl radical; the latter has been proposed to abstract a hydrogen atom from aminoethanol (Scheme 1: **1** → **2**).[4] There are some indications that the protein-associated radical could participate in that step as well.[5] On the basis of experimental findings, two basic pathways have been proposed for the subsequent rearrangement of **2**: an amine-migration route (**2** → **4**) and an amine-dissociation pathway (**2** → **10**).[6,7] Both routes yield eventually the same final products, i.e., ethanal, **11**, and ammonia; **11** is formed by reabstraction of a hydrogen atom from 5′-deoxyadenosine, thus regenerating the adenosyl-Cob(III)alamin and closing the catalytic cycle.

Our recent computational exploration[8] of several possible rearrangement mechanisms for this biologically important reaction proved quite useful in that numerous

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(1) The original name of the enzyme was ethanol deaminase (see ref 3), but later it was changed to ethanolamine ammonia lyase (EC 4.3.1.7).

(2) While the actual substrate is 2-aminoethanol, for the sake of shortness, it is abbreviated in the text as aminoethanol.

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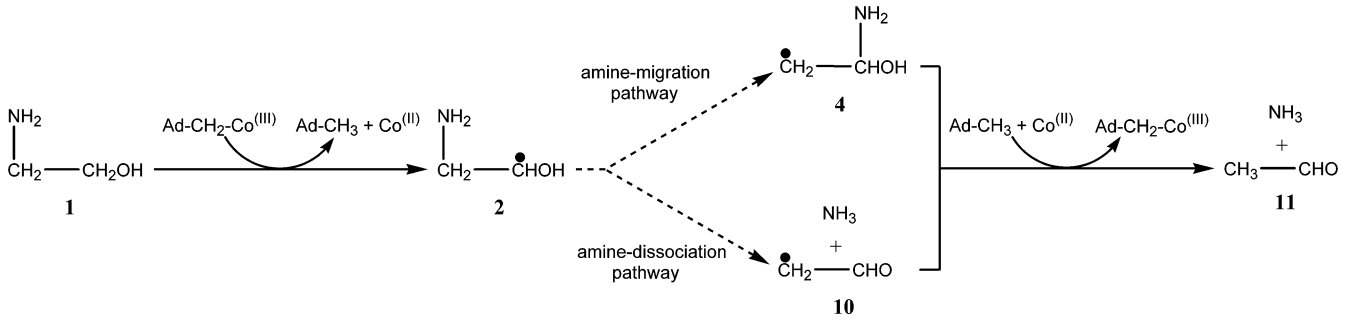
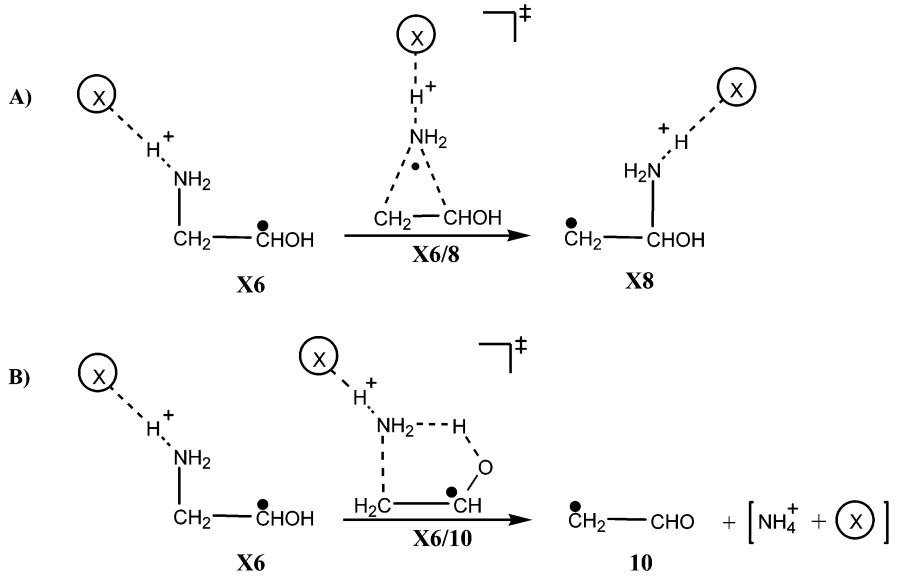
SCHEME 1. Possible Rearrangement Paths in the Deamination of Aminoethanol, 1, by Ethanolamine Ammonia Lyase[a]



[a] To enable a more straightforward comparison with a previous study (ref 8), the same structure labeling code is used here.

SCHEME 2. Partially Protonated Aminoethanol Radical 6 Serving as a Precursor for (A) NH_3 Migration and (B) NH_4^+ Elimination (X stands for different interacting groups)



conceivable pathways could be ruled out and only two rearrangements were indicated to meet the energetic requirements prevailing in the enzymatic process; these processes, commencing with a protonated substrate **6**, are depicted in Scheme 2 (note, that the computations reported in ref 8 do not include a catalytic auxiliary X). Clearly, any acceptable mechanism for ethanal formation, in the context of the ethanolamine ammonia lyase reaction, must be compatible with the rate constant for the product formation. Taking the value of $k_{cat} = 55\ s^{-1}$ for the ethanolamine ammonia lyase at 295 K,[9] the activation enthalpy for the rate-determining step can be derived from the Eyring equation. Assuming a reasonable range of activation entropy of 0−10 cal/mol·K,[10] the activation enthalpy falls into a range of 14.9−17.8 kcal/mol, respectively. Further, from the study of deuterium kinetic isotope effects, it was concluded that the rate-determining step in the overall reaction sequence corresponds to the hydrogen abstraction from the 5′-deoxyadenosine by the product radical.[11] The estimated energy barrier associated with that reaction step is equal to 15 kcal/mol at 298 K;[12] thus, any process with an activation enthalpy exceeding ca. 15 kcal/mol can be discarded as a potential step in the enzyme-catalyzed deamination of aminoethanol. Further, the previous exhaustive computations[8] demonstrated that all mechanistic variants involving unprotonated **2** were found to exceed considerably this upper energetic limit. In contrast, the activation enthalpy for the migration of an NH_3 group (Scheme 2A, but without X) in the fully protonated aminoethanol radical **6** was computed to be the lowest (10.4 kcal/mol) of the rearrangement barriers investigated in that study.[8] Similarly, elimination of NH_4^+ from **6** (Scheme 2B, but without X) has a comparable energy requirement of 10.6 kcal/mol. Therefore, both mechanisms could take place under the enzymatic conditions. Indeed, these findings confirm Radom's and Golding's seminal hypothesis[13−15] that protonation of a migrating group reduces signifi-

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cantly the energy barrier to the extent that they get close to the energetics pertinent to enzyme catalysis.

The present computational investigation aims at (i) further distinguishing between the two mechanistic scenarios identified as the most probable rearrangement pathways in the previous computational study[8] and (ii) to refine the mechanistic picture of the reaction. To this end we will apply the more realistic concept of *partial* protonation of a migrating group introduced earlier by Smith, Golding, and Radom.[13−15] By the time we were about to complete this work, we became aware of a related study by Radom and co-workers that addressed the same problem.[16] However, there are some distinct differences in the two studies; while we focus on distinguishing between the direct migration of a partially protonated amino group versus its elimination, in Radom's study the emphasis is to identify and characterize computationally the catalytic site involved in the rearrangement process. Further, since a push−pull mechanism, as proposed by Radom and co-workers in the case of diol dehydrase[17] and employed in the study of ethanolamine rearrangement catalyzed by ethanolamine ammonia lyase as well,[16] seems to play a crucial role in the catalytic activity of ethanolamine ammonia lyase, this mechanistic variant was also included in the present study. In contrast to the previous work,[16] we chose a model that is more appropriate for mimicking the actual amino acids. We note in passing a difference between the ethanolamine ammonia lyase and the diol dehydrase in that for the latter system K^+ ion was shown to play a crucial role in reducing the reaction barrier due to its interaction with the migrating group.[17,18]

While for the *fully* protonated substrate **6** the activation enthalpy difference for the two competing reactions as depicted in Scheme 2 (without X) is rather small (0.2 kcal/mol) in contrast to those involving uncharged **2**, it can be expected that *partial* protonation is likely to discriminate between the two pathways. It is this aspect to which we will pay attention in the present work. Inclusion of solvent, e.g., water molecules, in the calculations is not warranted on the ground that hydrogen exchange has not been observed in vitamin B_{12}-dependent rearrangements.[19] Thus, any continuum solvent model is not likely to provide more reliable data than the gas-phase calculations presented herein since the choice of dielectric constant needed for such calculations is ambiguous. As well, it was shown that the dielectric constant and physical environment of an enzyme's active site are often closer to those in the gas phase than in bulk solution.[20] Further, it was shown that the protein backbone can substantially influence a local pH.[21] In several B_{12}-dependent enzymes the amino acid sequence of the enzyme's active site were found to contain Asp and His residues,[22] which might serve as proton donors. In the case of the related methylmalonyl-CoA mutase catalyzed rearrangement, it was concluded, based on mutagenesis studies, that His-244 acts as a Brønsted acid.[23] For example, mutation of His-244 from the wild-type enzyme into Gly-244 in the mutant led to ca. 300-fold lowering in the catalytic efficiency of the enzyme. In addition, the crystal structure of methylmalonyl-CoA mutase indicated that His-244 is within hydrogen-bonding distance to the carbonyl oxygen of the carbonyl-CoA moiety.[24,25] Interestingly, computational studies by Wetmore et al.[26] confirmed the experimental findings that His most likely partially protonates the substrate. However, recent QM/MM studies indicated that besides His-244, two additional amino acids (i.e., Gln-197, Tyr-89) might contribute as well in reducing the activation barrier.[27] In the case of glutamate mutase it was shown by mutagenesis studies that Glu-171 plays a role of a catalytic auxiliary,[28] which was confirmed by theoretical studies.[29] While the amino acid sequence of the ethanolamine ammonia lyase has been determined,[30] the X-ray structure of the enzyme is not yet known. Consequently, no information is available which amino acids are relevant for a protonation of **1** (or **2**) and how precisely they are positioned in the active site. Therefore, in the present theoretical study several catalytic auxiliaries will be considered (Scheme 2), including some model systems for His (X = CH_2NH, imidazole) and Asp/Glu (X = HCOOH, CH_3COOH). Further, the question of the catalytic auxiliary(ies) that interact synergistically with the substrate in the enzyme's active site will be addressed, and we will demonstrate that it is this interplay of both a partial protonation of the NH_2 group and a deprotonation of the OH group of **1** (or **2**) that results in an activation enthalpy compatible with the experimental findings.

Computational Methods

All calculations were performed with the GAUSSIAN 98 suite of programs[31] using the DFT and QCISD approaches. The use of the DFT formalism was a natural choice because of the balance between accuracy and computational time required by the calculations. The B3LYP functional was employed.[32,33] Geometry optimizations were performed with Pople's polarized double-ζ 6-31G* basis set.[34] To characterize the optimized structures, frequency analysis has been per-

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formed at the same level of theory. Minima were characterized by the absence of imaginary vibrational frequencies, while transition structures exhibited one imaginary frequency. Computations of reaction pathways, i.e., intrinsic reaction coordinate (IRC) calculations and relaxed scans of the potential energy surface (PES), were carried out at the same level of theory.

Because the B3LYP method occasionally performs quite unsatisfactorily in the case of the reaction enthalpy evaluations,[35] single-point calculations and in some cases even geometry reoptimizations were performed at the QCISD level of theory using Dunning's correlation-consistent double-ζ basis set cc-pVDZ[36] in order to obtain more accurate energetic profiles of the reactions in question. Since the geometries of transition structures are not always properly described at the B3LYP level of theory,[37] a well-known underestimation of the barriers at the B3LYP level of theory is expected to result. The CBS−RAD (QCISD, B3LYP) method[38] has not been applied since the computational cost would be even higher and the energetic picture of the overall rearrangement pathways would not change substantially (see further below in text). Finally, a uniform scaling factor of 0.9806 was used for the zero-point energy (ZPE) corrections calculated at the B3LYP level of theory;[39] relative energies (given in kcal/mol) discussed in the text correspond to the enthalpies at 298 K obtained at the QCISD level of theory (SP calculations),[40] unless specified otherwise. Electronic energies, ZPEs, and the enthalpies of stationary points are quoted in the Supporting Information.

The structure labeling code used in our previous study[8] for the direct migration of an NH_3 group (e.g., TS label: **6/8**) and the elimination of NH_4^+ (e.g., TS label: **6/10**) was employed with a difference that herein the structures of analogous rearrangement types (migration vs elimination) for different protonating groups HX^+ are described by similar labels distinguished only in the prefix X (more on structure labeling see in the Appendix of the Supporting Information). As to the formal description of the reactions depicted in Scheme 2, partial protonation and its consequences for the two competing processes can be viewed as a reaction of **2** with a Brønsted acid HX^+ in which, after formation of **6**, the conjugated base X remains interacting with **6** throughout the whole reaction; alternatively, one may describe the rearrangement in terms of a reaction in which **2** is "sharing" a proton with HX^+. In the computations reported next we are using the former formalism, i.e., the rearrangement commences with **6** that itself remains interacting via sharing its proton with X.

TABLE 1. Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel,\ 0\ K}$) and 298 K ($H_{rel,\ 298\ K}$) of the Stationary Points on the PES of 6 Interacting with NH_3

	B3LYP/6-31G*		QCISD/cc-pVDZ// B3LYP/6-31G*		QCISD/cc-pVDZ[c]	
	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$
N6$_1$	0.0	0.0	0.0	0.0	0.0	0.0
N6$_1$/8	12.8	13.1	19.9	20.2	20.2	20.5
N8	2.0	2.0	-1.0	-1.0	-0.6	-0.6
N6$_2$	-0.4	-0.7	-0.9	-1.2		
N6$_2$/10	17.3	17.5	23.1	23.3		
N6[b]	-5.5	-5.3			-5.5	-5.3
N6/8[b]	12.6	12.8				
N8[b]	3.0	3.2				

[a] For electronic energies, ZPEs, and enthalpies, see Table 1S in the Supporting Information. [b] Structures contain an OH group orientation pointing away from the NH_3 group (see Scheme 4); thus, elimination of NH_4^+ is not possible for these conformers. [c] Data to be discussed in the section Influence of the OH Group Conformation on the Migration.

TABLE 2. Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel,\ 0\ K}$) and 298 K ($H_{rel,\ 298\ K}$) of the Stationary Points on the PES of 6 Interacting with H_2O

	B3LYP/ 6-31G*		QCISD/cc-pVDZ// B3LYP/6-31G*		QCISD/ cc-pVDZ[c]	
	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$
O6$_1$	0.0	0.0	0.0	0.0	0.0	0.0
O6$_1$/8	9.9	10.0	16.6	16.7	16.9	16.9
O8	2.8	2.7	-0.1	-0.2	0.4	0.3
O6$_2$	0.2	0.3	0.2	0.3		
O6$_2$/10	14.6	14.7	18.7	18.8		
10·NH_4^+·H_2O	-17.1	-16.7	-15.9	-15.4		
10 + NH_4^+·H_2O	1.9	2.7	1.4	2.2		
O6[b]	-4.9	-4.8			-5.0	-4.8
O6/8[b]	9.6	9.7				
O8[b]	2.6	2.7				

[a] For electronic energies, ZPEs, and enthalpies, see Table 2S in the Supporting Information. [b] Structures contain an OH group orientation pointing away from the NH_3 group (see Scheme 4); thus, elimination of NH_4^+ is not possible for these conformers. [c] Data to be discussed in the section Influence of the OH Group Conformation on the Migration.

Results and Discussion

Migration vs Elimination.

(1) Hydroxonium and ammonium ions as protonating groups. As the amino acids Asp, Glu, and His contain in their proton donor part either an oxygen or a nitrogen atom, we first investigated the protonation of **2** and the ensuing rearrangements of **6** using NH_4^+ and H_3O^+ as Brønsted acids.

As expected, with NH_4^+ both transition structures, **N6$_1$/8** and **N6$_2$/10**, are energetically more demanding than when the stronger Brønsted acid H_3O^+ serves as a proton donor (Tables 1 and 2). Comparing structural details of the transition structures for the reactions with the two protonating groups (see Figure 1), it is obvious that in the TSs proton transfer from H_3O^+ is more advanced in comparison to the analogous structures with NH_4^+ (see, e.g., the H···X distances in **N6$_1$/8** and **O6$_1$/8** correspond to 1.831 vs 1.898 Å). Correspondingly, the N···H distance is larger for N···H···X when X = NH_3 (1.055 Å) than for X = H_2O (1.030 Å).

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(40) The QCISD enthalpies at 298 K were computed as fallows: ΔH (QCISD; 298 K) = ΔH (QCISD; 0 K) + ΔH (B3LYP; 298K) − ΔH (B3LYP; 0 K), where enthalpies at 0 K include the sum of the electronic energy and ZPE corrections scaled by 0.9806.

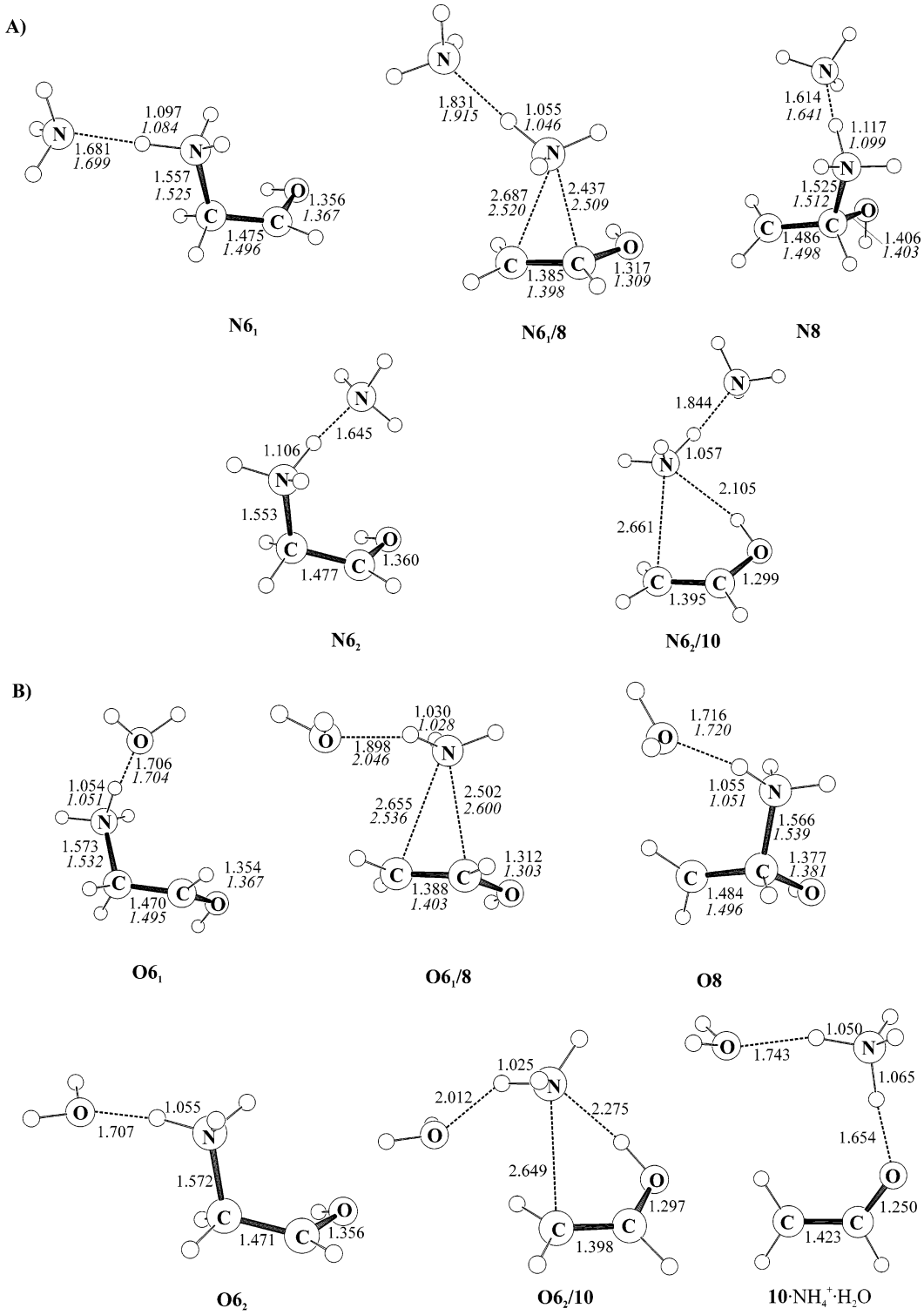
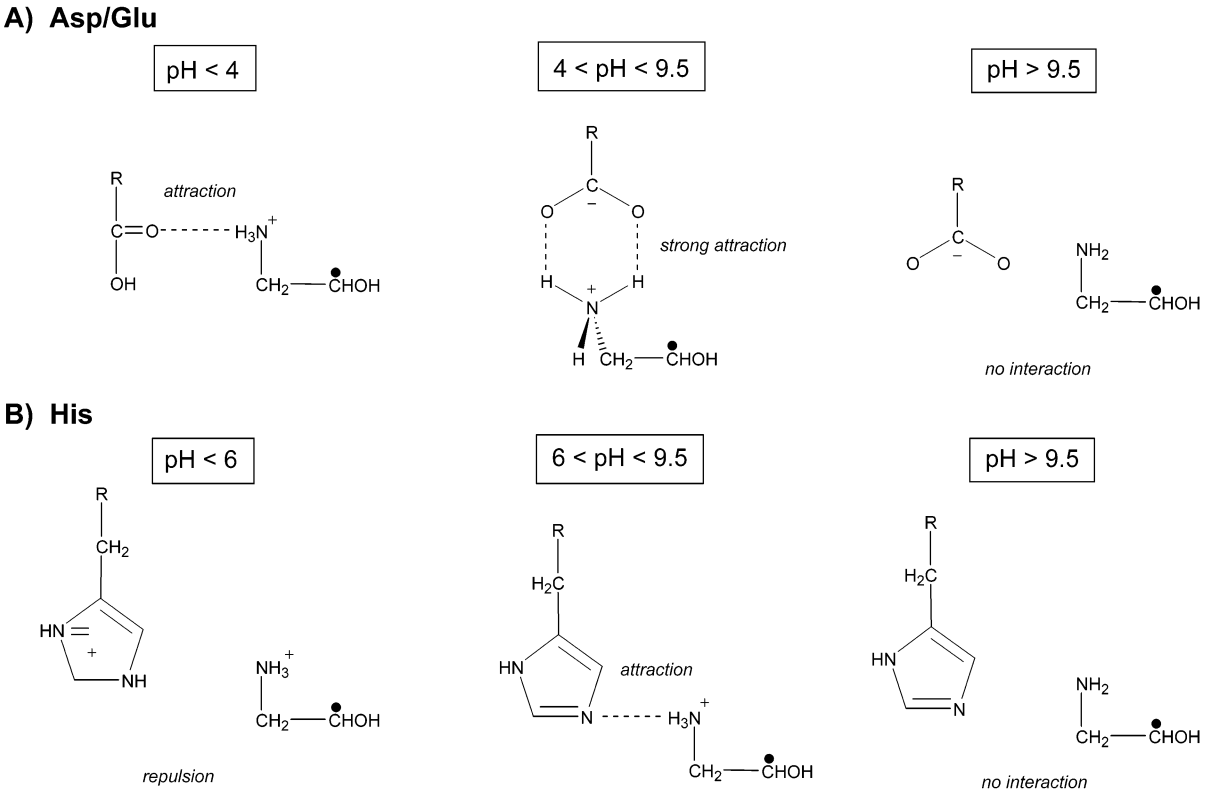


FIGURE 1. Optimized geometries of stationary points relevant for the rearrangements of partially protonated aminoethanol radical **6** interacting with (A) NH_3 and (B) H_2O (bond lengths are given in Å; B3LYP results in roman and QCISD in italics).

Common to both Brønsted acids is that the transition structures **N6$_1$/8** and **O6$_1$/8** for a migration of the protonated amino group are energetically less demanding than for the elimination of NH_4^+ via **N6$_2$/10** and **O6$_2$/10**. The activation enthalpy difference between these two transition structures **X6$_1$/8** and **X6$_2$/10** varies slightly: it is smaller in the case of H_3O^+ (2.1 kcal/mol) than for NH_4^+ (3.1 kcal/mol). Even though the differences in activation enthalpy between these two reactions are still rather small, they are much more pronounced than in the previous study (0.2 kcal/mol),[8] where only full protonation of the substrate **2** was taken into account. Further, in the H_3O^+ initiated reaction, a complex between the emerging ethanal radical, **10**, NH_4^+ and H_2O was found to exist on the potential energy surface (PES; see Figure 1B). The energy requirement for its dissociation to-

SCHEME 3. Different Interaction Modes between the Amino Acid and the Substrate Depending on Assumed pH Values in the Active Site: (A) Asp/Glu and (B) His



liberate a free intermediate **10** is equal to 17.6 kcal/mol, thus making this pathway even less probable. However, formation of such a product complex can be bypassed since the enzyme could pull out NH_4^+ upon its formation. In any case, the computational data obtained for the rearrangements of **2** with the crude model systems NH_4^+ and H_3O^+ clearly indicate that *partial* protonation of the substrate results in an energetic discrimination between the two competing rearrangements. Next, we will check if this encouraging trend continues by employing improved amino acid-model systems.

(2) Active site—What is the most probable pH and what are its mechanistic consequences?

The precise pH of a reaction environment around an enzyme's active site is difficult if not impossible to predict,[41] even if the X-ray structure of the enzyme were known. Nevertheless, depending on the nature of the amino acids present in the active site the pH can be approximated provided the effective dielectric constant is known. As mentioned, deuterium labeling experiments suggest that solvent molecules do not have access to the active site; consequently, the effective dielectric constant depends mostly on the amino acids. However, as acidity is also affected by the molecular architecture of the actual environment (orientations of the amino acids, the protein's permanent and induced dipoles),[42] due to the absence of an X-ray structure, an accurate prediction of the local pH is not possible in the present case. Therefore, the discussion will be limited to several assumed pH ranges, determined by the acidity of the substrate and the amino acids Asp, Glu, and His (Scheme 3). Aminoethanol **1** behaves as a base having a pK_a of 9.45 for its conjugated acid. For radical **2** a similar pK_a can be reasonably assumed, as substantiated by high-level ab initio calculations for the proton affinities of aminoethanol **1** and its radical **2**, which are identical.[16] If the pH in the active site approximates the one prevailing under physiological conditions (pH $\sim$ 7.5),[6,7,43] most of the substrate **2** exists in its fully protonated form. However, partial rather than full protonation might well result through the interaction of the **2**/**6** couple with amino acid residues, which can serve as proton donors or as buffer reagents. As a result, substrate **2** captured in the active site would not be completely "free" but would interact with the protein backbone; clearly, such a refined picture resembles more closely enzyme-catalyzed reactions, where an enzyme through interaction with a substrate lowers the transition structure energy and thus serves as a catalyst.[44]

(a) Asp/Glu as protonating agents (Scheme 3A). Assuming the amino acids Asp or Glu act as proton donors, the pH regimes can be meaningfully divided in three regions, which are determined by the pK_a values of the Asp/Glu side chains[45,46] and the pK_a of the substrate. In the case of related glutamate mutase it was confirmed that Glu-171 acts as a catalytic auxiliary.[28,29]

(41) For the sake of shortness, it is the pH of the reaction environment around the enzyme's active site which is meant when the phrase "pH in the active site" is used. For a superb discussion of this and related aspects, see: Barril, X.; Alemán, C.; Orozco, M.; Luque, F. J. *Proteins* **1998**, *32*, 67.

(42) Warshel, A. *Biochemistry* **1981**, *20*, 3167.

(43) Bandarian, V.; Reed, G. H. *Biochemistry* **1999**, *38*, 12394.

(44) (a) Pauling, L. *Chem. Eng. News* **1946**, *24*, 1375. (b) Pauling, L. *Am. Sci.* **1948**, *36*, 51.

At pH < 4, amino acids exist in their nondissociated form (RCOOH) while substrate **2** is protonated. Nevertheless, an interaction between the amino acid and the substrate is possible through a H-bond between the basic carbonyl oxygen of the COOH group and a hydrogen from the NH_3^+ group. While that kind of interaction is expected to stabilize the structure due to a better redistribution of charge, such a low pH in the active site is not common for enzymes; however, as it cannot be discarded a priori, this scenario was investigated by computational means.

In the pH regime 4 < pH < 9.5, both amino acids Asp/Glu exist in their carboxylate forms ($RCOO^-$) while the substrate is still protonated. Therefore, a "salt bridge"-like interaction exists between the two charged species. As a consequence, the migrating NH_2 group of **2** is only weakly protonated, and this results in a destabilization of the corresponding transition structures. However, as this pH range is common for biological systems it presents a realistic pH scenario in the active site of the enzyme in question.

At pH > 9.5, the substrate is not protonated and the amino acids exist in their dissociated forms. An interaction between the two basic sites certainly would not contribute to a stabilization of the transition structure. As any process involving *neutral* **2** requires unrealistically high activation enthalpies,[8] a further investigation of such an interaction was not taken into account. However, interactions of basic auxiliaries with the OH group of **2** will be addressed.

(b) His as a protonating agent (Scheme 3B). His is a common amino acid, which serves as a proton buffering agent in numerous biological systems because of its pK_a value of 6, which is close to a physiological pH. For example, in the case of the methylmalonyl-CoA mutase-catalyzed rearrangement it was concluded that His-244 is the very amino acid that partially protonates a substrate and, therefore, lowers the transition barrier.[23−26]

If pH < 6, His and aminoethanol radical **2** are in their N-protonated forms, and consequently, repulsion is expected as a net effect. Clearly, such a scenario need not to be addressed further.

In the pH regime 6 < pH < 9.5, the N(3) atom of His is not protonated while the substrate **2** is expected to be protonated. Here, a H-bond between N(3) of His and a proton from the NH_3 group of **6** is likely to stabilize a transition structure via charge delocalization. As mentioned, this particular pH range is common for biological systems, and a computational investigation of this scenario was undertaken.

At pH > 9.5, both the substrate and His are not protonated. Since no stabilization occurs and all barriers involving **2** are high,[8] a further investigation was not pursued.

(3) His Serving as a Proton Donor.

For the interaction of the substrate with His we first used the most simple imine (methanimine, CH_2═NH) as a model system. Despite its limitations, methanimine is structurally closer to His than NH_3 (e.g., the hybridization of the basic nitrogen atoms that is crucial for the reaction). We note, however, that the proton affinity (PA) of methanimine (203.8 kcal/mol) is similar to the PA of ammonia (204.0 kcal/mol), and both differ considerably from the PA of His (236.0 kcal/mol);[47] consequently, an even closer model system for His had to be introduced (see further in the text).

TABLE 3. Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel, 0\ K}$) and 298 K ($H_{rel, 298\ K}$) of the Stationary Points on the PES of 6 Interacting with CH_2═NH (Mi) Which Serves as the Simplest His Model System

	B3LYP/6-31G*		QCISD/cc-pVDZ// B3LYP/6-31G*		QCISD/cc-pVDZ[c]	
	$H_{rel, 0\ K}$	$H_{rel, 298\ K}$	$H_{rel, 0\ K}$	$H_{rel, 298\ K}$	$H_{rel, 0\ K}$	$H_{rel, 298\ K}$
Mi-6$_1$	0.0	0.0	0.0	0.0	0.0	0.0
Mi-6$_1$/8	12.4	12.6	19.8	20.0	20.0	20.3
Mi-8	2.1	2.2	-0.8	-0.8	-0.4	-0.4
Mi-6$_2$	0.0	0.0	0.0	0.0		
Mi-6$_2$/10	16.8	17.0	22.3	22.5		
Mi-6[b]	-5.4	-5.2			-5.5	-5.2
Mi-6/8[b]	12.1	12.4				
Mi-8[b]	2.9	3.1				

[a] For electronic energies, ZPEs, and enthalpies see Table 3S in the Supporting Information. [b] Structures contain an OH group orientation pointing away from the NH_3 group (see Scheme 4); thus, elimination of NH_4^+ is not possible for these conformers. [c] Data to be discussed in the section Influence of the OH Group Conformation on the Migration.

As discussed above, only a pH range of 6−9.5 needs to be taken into consideration. In this pH regime the strongest attraction between the substrate and His is expected to exist between the protonated amino group from the substrate and the imidazole sp^2-hybridized nitrogen atom from His (see Scheme 3B).

In fact, the trends already observed with the most simple protonating groups (H_3O^+, NH_4^+) exist in the case of the His model methanimine (**Mi**) as well: *migration* of a partially protonated amino group is energetically less demanding than *elimination* of the ammonium ion. According to the calculations, the transition structure **Mi-6$_1$/8** is 2.5 kcal/mol lower in energy than its **Mi-6$_2$/10** counterpart (Table 3). The stabilization of the former transition structure is most probably achieved through the formation of a relatively stable planar enol radical cation moiety.[48] In contrast, in the **Mi-6$_2$/10** transition structure, the enol is distorted from planarity (Figure 2) and already shows a trend of ethanal radical formation; for example, the C−O bond is shorter, while the C−C bond of **Mi-6$_2$/10** is longer than corresponding bonds in **Mi-6$_1$/8**.

Here, a comment on the computed values for the activation enthalpies seems warranted: for the migration of a partially protonated amino group in the system **6**/CH_2═NH this barrier height amounts to 20.0 kcal/mol, exceeding the upper limit value (ca. 15 kcal/mol). However, a final answer whether an interaction of His with the substrate is of any relevance on the migration

(45) (a) Dawson, R. M. C.; Elliott, D. C.; Elliott, W. H.; Jones, K. M.; *Data for Biochemical Research,* 3rd ed.; Oxford Science Publication, Oxford, 1986. (b) Perrin, D. D.; Dempsey, B.; Serjeant, E. P. *pK_a Prediction for Organic Acids and Bases;* Chapman and Hall Publishers: London, 1981.

(46) pK_a (Asp) = 3.9, pK_a (Glu) = 4.1, taken from ref 45.

(47) *NIST Chemistry WebBook;* Linstrom, P. J., Mallard, W. G., Eds.; NIST Standard Reference Database Number 69; National Institute of Standards and Technology, Gaithersburg, MD, July 2001 (http://webbook.nist.gov).

(48) (a) Smith, B. J.; Tho, N. M.; Bouma, W. J.; Radom, L. *J. Am. Chem. Soc.* **1991**, *113*, 3, 6452. (b) Turecek, F.; Cramer, C. J. *J. Am. Chem. Soc.* **1995**, *117*, 12243.

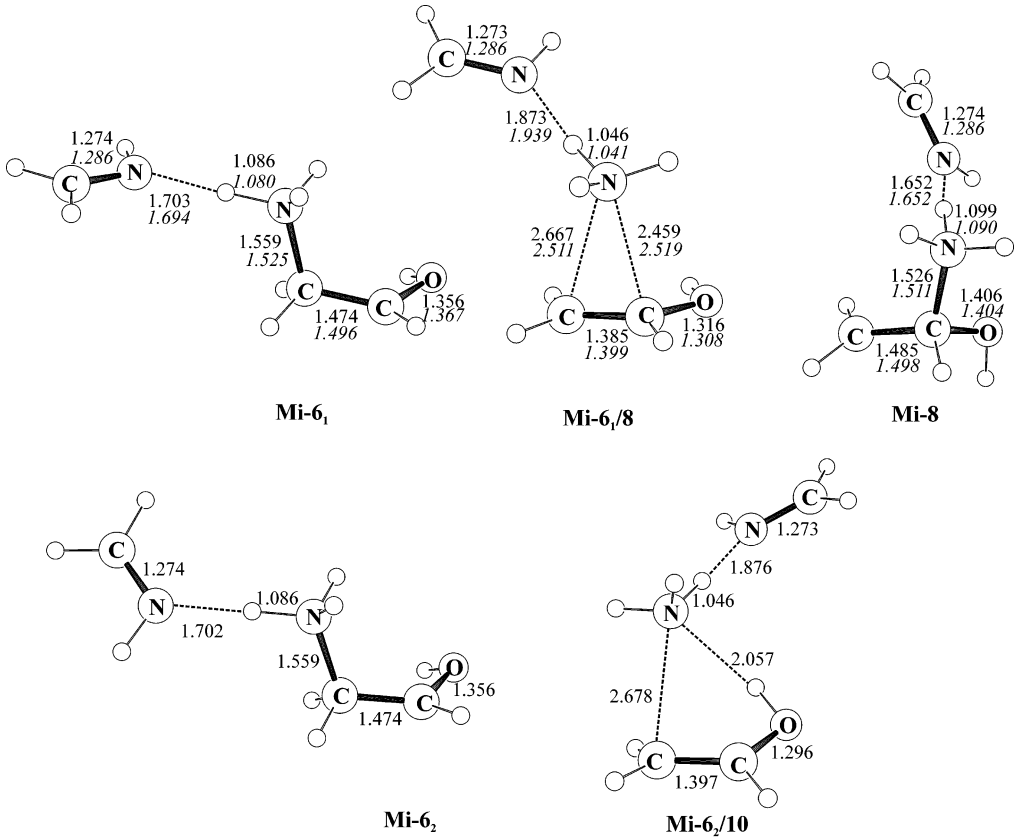


FIGURE 2. Optimized geometries of stationary points relevant for the rearrangements of partially protonated aminoethanol radical **6** interacting with CH_2=NH, which serves as a His model system (bond lengths are given in Å; B3LYP results in roman and QCISD in italics).

pathway can be given only after an even more reliable model system for His has been considered (see further in the text). Nevertheless, the migration of a partially protonated amino group is once more favored as compared to the elimination of NH_4^+.

(4) Asp/Glu serving as proton donors.

Since both amino acids Asp and Glu have similar structures and comparable acidities, we first used formic acid (**Fo**) as a rather crude model to represent both of them (Figure 3). For the sake of shortness, in the text we will refer only to Asp. Two pH ranges will be discussed in some detail.

At pH < 4 Asp exists in its nondissociated form and the substrate **2** is protonated. Again, the activation enthalpy for the migration involving **Fo-6$_1$/8** is 2.8 kcal/mol lower than for the elimination proceeding through the **Fo-6$_2$/10** transition structure (Table 4; note that the rearrangements do not commence from the same conformer **Fo-6**). While the activation enthalpy for the migration of a protonated amino group with 16.2 kcal/mol falls into the range of required activation enthalpies (ca. 15 kcal/mol), the assumed pH < 4 is not common for biological systems.

In a pH regime of 4−9.5 Asp is deprotonated while the substrate still prefers the protonated form. Since solvent molecules do not have access to the active site of ethanolamine ammonia lyase,[19] the overall "neutral" H-bonded complexes, which can be viewed as counterparts to "salt bridges", obtained as the stationary points on the PES are believed to be more stable in the gas phase. In fact, computational studies on similar "salt bridge" complexes in biological systems showed that neutral complexes with double H-bonds between the two charged building blocks are more stable in the gas phase than their zwitterionic counterparts; however, the latter are clearly favored in an aqueous environment.[49]

Here, the **Fo-6$_1$** structure can be interpreted as **2** interacting with the formic acid; thus, in the transition structure **Fo-6$_1$/8** a required protonation of the migrating group NH_2 cannot occur, and thus, no stabilization of TS will take place. Further, the migrating group does not have a net charge and is no longer a good leaving group either (for example, the C−N bonds of **Fo-6$_1$/8** are shorter than in the analogous **Fo-6$_1$/8** transition structure; Figure 3); thus, the transition structure **Fo-6$_1$/8** is energetically extremely unfavored (Table 5: 80.2 kcal/mol), making this reaction highly improbable. Finally, the **Fo-6$_1$/8** transition structure can be described in terms of a migration of the amino group in aminoethanol radical **2** only weakly interacting with the HCOOH moiety. However, as already shown and discussed in detail,[8] rearrangement of unprotonated aminoethanol radical

(49) (a) Zheng, Y.-J.; Ornstein, R. L. *J. Am. Chem. Soc.* **1996**, *118*, 11237. (b) Jockush, R. A.; Lemoff, A. S.; Williams, E. R. *J. Am. Chem. Soc.* **2001**, *123*, 12255. (c) Freitas, M. A.; Marshall, A. G. *Int. J. Mass. Spectrom.* **1999**, *182/183*, 221. (d) Schnier, P. D.; Price, W. D.; Jockusch, R. A.; Williams, E. R. *J. Am. Chem. Soc.* **1996**, *118*, 7175. (e) Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 12840.

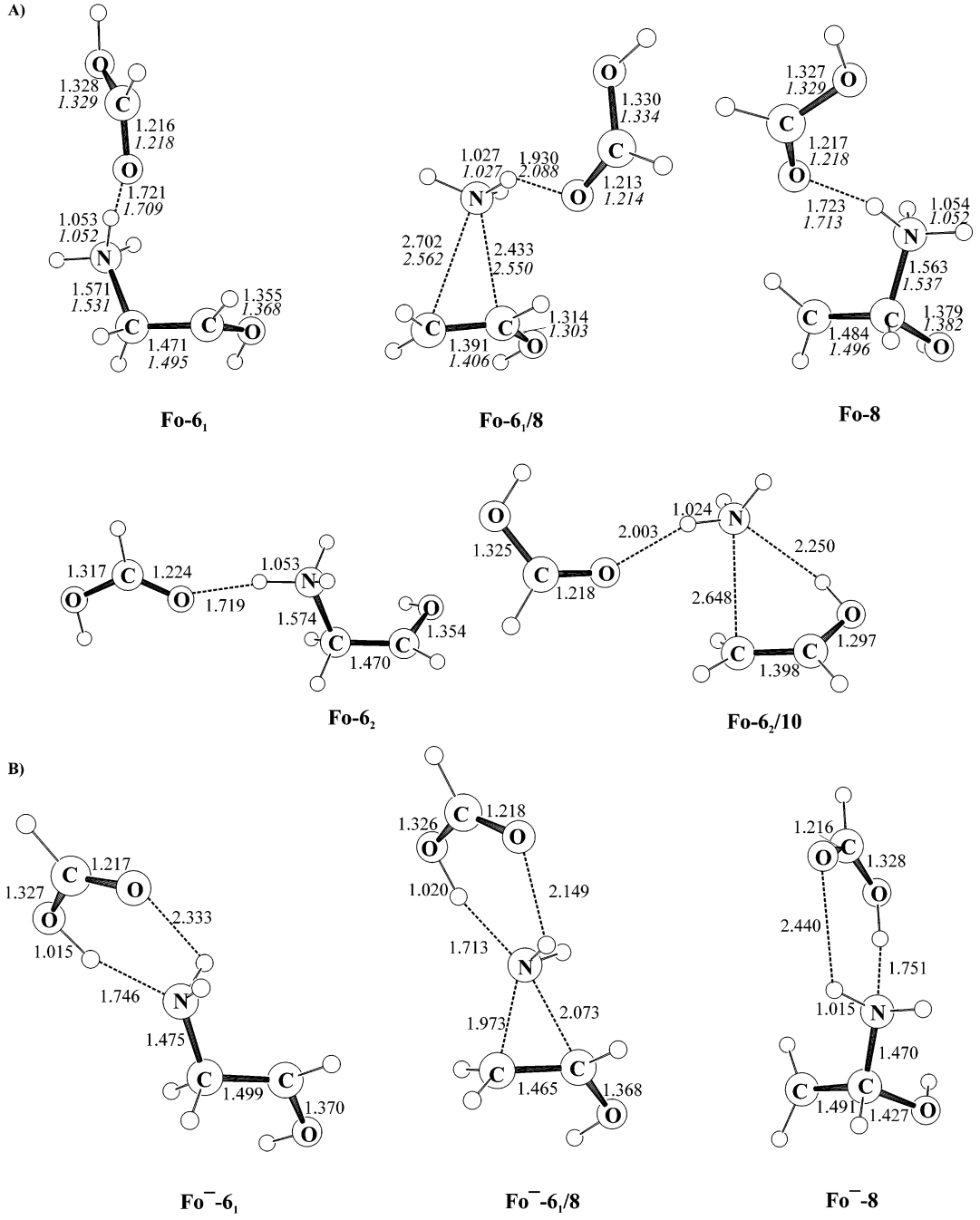


FIGURE 3. Optimized geometries of stationary points relevant for the rearrangements of partially protonated aminoethanol radicals **6** interacting with Asp/Glu model systems: (A) HCOOH acid and (B) $HCOO^-$ (bond lengths are given in Å; B3LYP results in roman and QCISD in italics).

2 is extremely demanding energetically, and consequently, the new findings do not come as any surprise.

A Preliminary Résumé. The major finding of the first part of this computational study of the aminoethanol rearrangement, including different models for a partial substrate protonation, clearly points to a discrimination between the two most likely mechanisms (Scheme 2: direct NH3 migration, **6** → **8**, vs NH_4^+ elimination, **6** → **10**).[8] As a result, *migration of the partially protonated amino group* is shown to be energetically less demanding in all investigated cases in comparison to the elimination process.

In the recently published paper, Radom and co-workers[16] arrived at the same conclusion. Employing isodesmic reactions for the hydrogen abstraction from the 5′-deoxyadenosine model system by the putative product radicals **8** and **10**, the authors concluded that the migration **6** → **8** constitutes a more favorable scenario since hydrogen abstraction from 5′-deoxyadenosine by **8** is calculated to be exothermic (−1.2 kcal/mol), while the same reaction, where **10** abstracts a hydrogen atom, is quite endothermic (6.2 kcal/mol). Possible barriers associated with both process have not been reported.[16]

(1) Influence of the OH group conformation on the migration aptitude.

SCHEME 4. Different OH Group Orientations in the (partially) Protonated Aminoethanol Radical 6 and Their Influence on the Migration Pathway

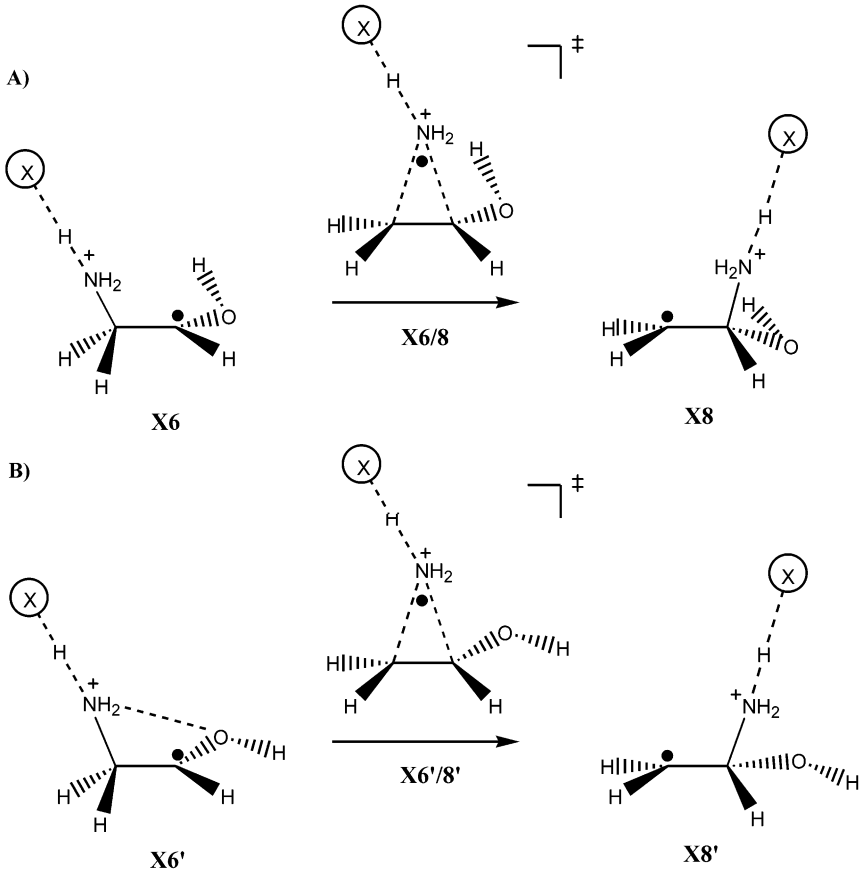


TABLE 4. Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel, 0\ K}$) and 298 K ($H_{rel, 298\ K}$) of the Stationary Points on the PES of 6 Interacting with HCO_2H (Fo) Serving as the Simplest Asp/Glu Model System

	B3LYP/6-31G*		QCISD/cc-pVDZ// B3LYP/6-31G*		QCISD/cc-pVDZ[c]	
	$H_{rel, 0\ K}$	$H_{rel, 298\ K}$	$H_{rel, 0\ K}$	$H_{rel, 298\ K}$	$H_{rel, 0\ K}$	$H_{rel, 298\ K}$
Fo-6$_1$	0.0	0.0	0.0	0.0	0.0	0.0
Fo-6$_1$/8	9.6	9.8	16.0	16.2	16.3	16.5
Fo-8	2.5	2.5	-0.3	-0.4	0.1	0.1
Fo-6$_2$	-0.6	-0.5	-0.5	-0.4		
Fo-6$_2$/10	14.6	14.9	18.3	18.6		
Fo-6[b]	-4.8	-4.8			-4.9	-4.8
Fo-6/8[b]	9.9	10.1				
Fo-8[b]	2.5	2.6				

[a] For electronic energies, ZPEs, and enthalpies, see Table 4S in the Supporting Information. [b] Structures contain an OH group orientation pointing away from the NH_3 group (see Scheme 4); thus, elimination of NH_4^+ is not possible for these conformers. [c] Data to be discussed in the section Influence of the OH Group Conformation on the Migration.

A realistic activation enthalpy for the migration process can be estimated only if the most stable conformer of the reactant is identified. For the migration **6** → **8** different conformations of the OH group are conceivable, out of which two extreme cases will be discussed in more detail (Scheme 4). One of the conformations might be crucial since the (partially) protonated aminoethanol radical **6** can be stabilized through an intramolecular H-bond interaction with the lone-electron pair of the OH group (Scheme 4B). Interestingly, in the analogous case of nonprotonated aminoethanol **2**, the orientation of the

TABLE 5. Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel, 0\ K}$) and 298 K ($H_{rel, 298\ K}$) of the Stationary Points on the PES of 6 Interacting with $HCOO^-$ (Fo^-)as a Dissociated Asp/Glu Model System

	B3LYP/6-31G*		QCISD/cc-pVDZ//B3LYP/6-31G*	
	$H_{rel, 0\ K}$	$H_{rel, 298\ K}$	$H_{rel, 0\ K}$	$H_{rel, 298\ K}$
Fo-6$_1$	0.0	0.0	0.0	0.0
Fo-6$_1$/8	72.8	72.7	80.3	80.2
Fo-8	-0.4	-0.4	-2.3	-2.2

[a] For electronic energies, ZPEs, and enthalpies, see Table 5S in the Supporting Information.

OH group toward the NH_2 group was found to be the most stable conformation due to the strong H-bonding between the hydrogen from OH and a nitrogen from the NH_2 group.[8,50] In the case of a H-bonding between the oxygen from the OH group and a hydrogen atom from the NH_3 group (Scheme 4B), one can expect that the stability of the OH group conformation will be dictated by the strength of the proton-donating group X. Therefore, we will explore the influence of an OH group conformation on the barrier for the migration employing different protonating groups.[51] Structures corresponding to the OH group oriented away from the NH_3 group will be denoted by a prime, e.g., **6′**, **8′**, etc.

(50) For a study on neutral aminoethanol, see: Silva, C. F. P.; Duarte, M. L. T. S.; Fausto, R. *J. Mol. Struct.* **1999**, *482−483*, 591.

(51) Conformational aspects for the interaction of **6′** with $HCOO^-$ will not be included due to the very high activation enthalpy associated with the migration pathway.

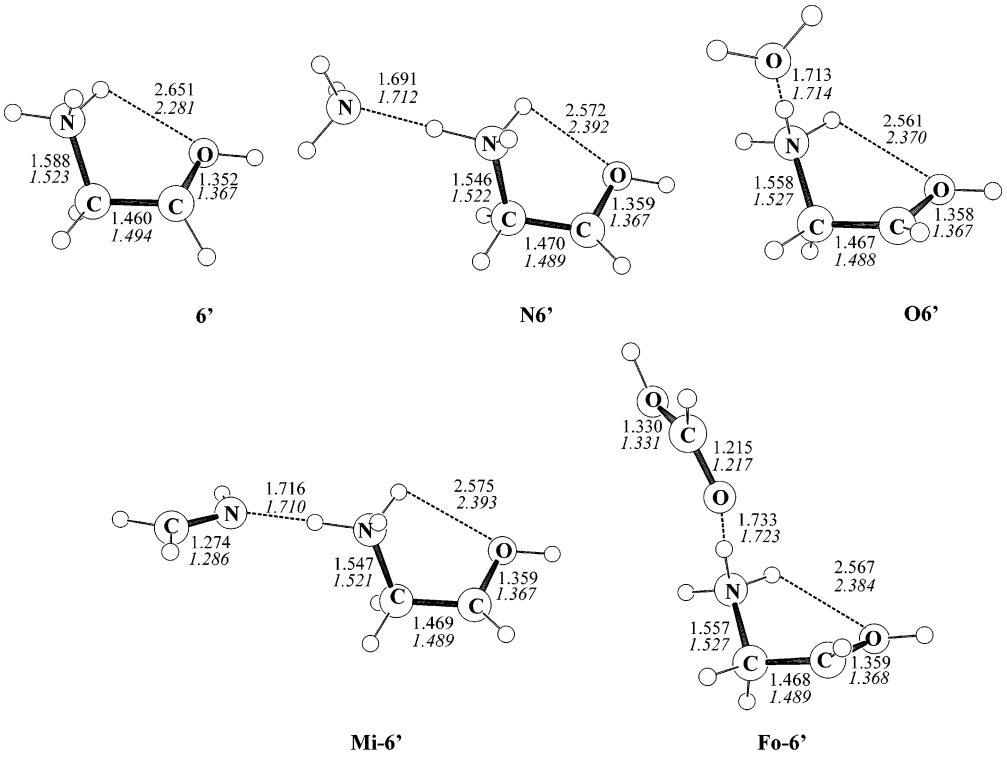


FIGURE 4. Optimized geometries of stationary points relevant for the rearrangements of (partially) protonated aminoethanol radicals **6′** with H-bond interaction (bond lengths are given in Å; B3LYP results in roman and QCISD in italics).

TABLE 6. Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel,\ 0\ K}$) and 298 K ($H_{rel,\ 298\ K}$) of the Stationary Points on the PES of Protonated Aminoethanol Radicals; Comparison between the Energetics of the Migration Pathways for Two Different Orientations of the OH Group in 6

	B3LYP/6-31G*		QCISD/cc-pVDZ	
	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$
6_1[a]	0.0	0.0	0.0	0.0
6_1/8[a]	4.7	4.9	10.2	10.4
8[a]	2.9	3.0	-0.3	-0.1
6[b]	-5.6	-5.6	-6.6	-6.6
6/8[b]	4.2	4.4	9.5	9.7
8[b]	2.8	2.8	0.5	0.5

[a] Energies taken from ref 8. [b] Structures contain an OH group orientation pointing away from the NH_3 group (see Scheme 4). For electronic energies, ZPEs, and enthalpies, see Table 6S in the Supporting Information.

To obtain even more reliable activation enthalpies for the migration rearrangements, the reoptimizations of **X6$_1$**, **X6$_1$/8,** and **X8** were performed at the QCISD/cc-pVDZ level of theory.

Let us first address the rearrangement of the fully protonated aminoethanol radical **6** (Scheme 4 but without X); here, the influence of the OH conformation should be most pronounced. According to the data in Table 6, conformer **6′** corresponds to the (global) minimum on the PES; this is due to a quite strong H-bond between the oxygen lone pair and a hydrogen atom from the NH_3 group (2.281 Å, QCISD geometry; Figure 4). The H-bond stabilization amounts to 6.6 kcal/mol. However, in line with the Curtin−Hammett principle it is irrelevant energetically whether the migration proceeds directly through **6′/8′** (16.3 kcal/mol) or sequentially **6′** → **6_1** (6.6 kcal/mol) and **6_1/8** (10.4 kcal/mol). The resulting overall activation energy as well as the product energies are almost identical (Table 6).

Since for the migration processes discussed previously we have located the transition structures commencing from a conformer in which a H-bond between the NH_2−H and OH groups does *not* exist, to estimate the energy demand for the overall migration process, the transition structures **X6′/8′** as well as the minima connected by the corresponding TSs for different protonating groups (X = H_2O, NH_3, CH_2NH, HCO_2H) were located at the B3LYP/6-31G* level of theory. Next, the geometry reoptimizations of the **X6′** structures were performed at the QCISD/cc-pVDZ level of theory in order to estimate the overall migration enthalpy as stated above.

Comparing the energies of the conformers **X6$_1$** and **X6′**, it can be seen that the stabilization gained through the H-bond interaction in the conformer **X6′** amounts to ca. 5 kcal/mol (Tables 1−4; QCISD/cc-pVDZ method), which implies that the overall migration pathways are ∼5 kcal/mol more demanding than previously stated. More precisely, for X = H_2O, the overall activation enthalpy for the migration is equal to 21.7 kcal/mol and for X = NH_3 it amounts to 25.8 kcal/mol. In the case of the small amino acid model systems the overall activation enthalpies change to 21.3 (X = HCOOH) and 25.5 kcal/mol (X = CH_2NH).

Not surprisingly, the activation enthalpy is lowest in the case of full protonation of **2**; as soon as deprotonation comes into play, the activation barrier increases substantially. However, even *partial protonation* renders the migration more favorable since the corresponding barriers for all investigated protonating groups are lower than for a completely deprotonated precursor **2**. At first sight, this statement is in disagreement with the conclu-

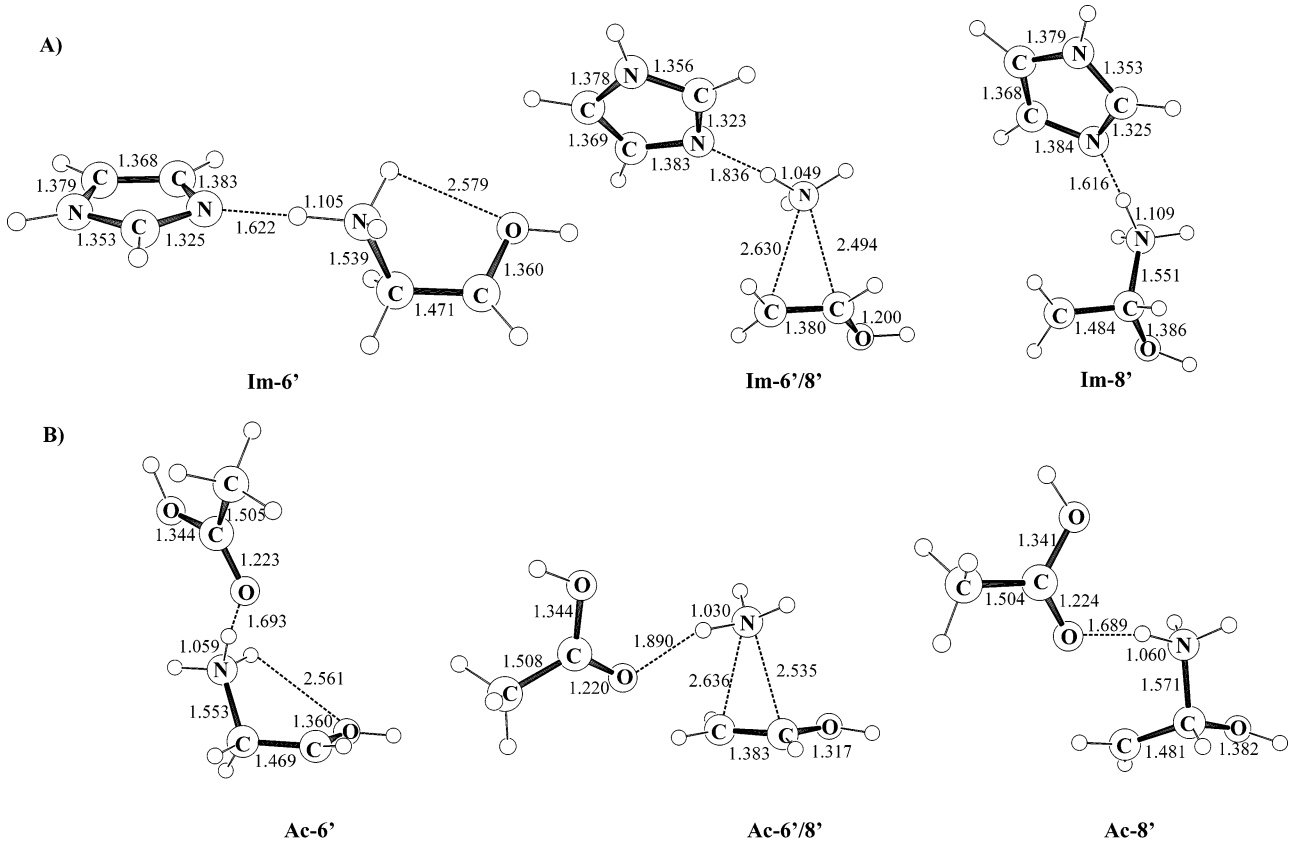


FIGURE 5. Optimized geometries of stationary points relevant for the rearrangements of partially protonated aminoethanol radical **6′** interacting with more realistic model systems for amino acids: (A) imidazole as His model system and (B) acetic acid as Asp/Glu model system (bond lengths are given in Å; B3LYP results).

sion derived by Radom and co-workers,[16] who concluded that partial deprotonation has an anticatalytic effect based on the activation energy comparison with the rearrangement of nonprotonated aminoethanol radical, **2** (23.6 kcal/mol; fragmentation−recombination mechanism[52]). However, for their particular case, the TS located does not commence from the most stable aminoethanol radical structure. In our previous study[8] we did locate that structure (denoted there as $\mathbf{2_2}$), which is 4.4 kcal/mol more stable than the aminoethanol radical conformer located by Radom and co-workers.[16] Keeping this in mind, the energy requirement for the rearrangement of the aminoethanol radical **2** amounts to 28 kcal/mol,[53] thus being higher than for any of the partially protonated precursors **X6**.

However, even if the partial protonation reduces the activation enthalpy compared to the nonprotonated case, all estimated migration enthalpies still exceed the activation-enthalpy upper limit determined experimentally as acceptable for an enzymatic catalysis (ca. 15 kcal/mol). One of the possible explanations could be related to the inaccuracy of the computational method chosen. However, concerning that topic a comparison with the data reported by Radom and co-workers[16] is warranted. The latter estimated the transition barrier **6′** → **8′** to be 15.7 kcal/mol (0 K) at the G3(MP2)-RAD(p) level of theory; this agrees pleasingly with our result (16.1 kcal/mol at 0 K). We therefore conclude that the computational method employed in our study produces data comparable in its quality to those obtained from the computationally more demanding G3 method. Further, we note that single-point calculations at the QCISD/cc-pVDZ level of theory on the B3LYP-optimized geometries provide very similar relative enthalpies as the data obtained by computationally more demanding geometry reoptimizations at the QCISD level of theory (see Tables 1−4). Clearly, for the cases that follow it is sufficient to perform only single-point calculations at the QCISD/cc-pVDZ level of theory since these data are of the same accuracy as those that would be obtained by the geometry reoptimizations at the same level of theory.

As the method chosen cannot be the cause for an overestimation of the activation barriers, we conclude that partial protonation alone does not sufficiently reduce the activation enthalpy; thus, some more refined mechanistic scenarios for the migration rearrangement have to be considered. However, before we discuss some of these mechanistic variants, to be on the safe side, we shall compute the migration pathways using some more appropriate model systems for the amino acids in order to eliminate a possible overestimation of the activation enthalpy caused by employing unrealistic model systems.

(52) In ref 8 this type of rearrangement is described as "dissociation−association mechanism".

(53) The barrier for the NH_2 dissociation process can be either estimated by locating a transition structure commencing from the (most) stable conformer $\mathbf{2_2}$ or, equivalently, calculating the activation enthalpy for the dissociation starting from the conformer in which the H-bond interaction does not exist and adding the energy difference between the two reactant conformers. Here, the latter method was used.

SCHEME 5. Push−Pull Mechanism for a Migration Pathway of 6 Involving a Partial Protonation of the NH_2 Group and a Partial Deprotonation of the OH Group of the Substrate

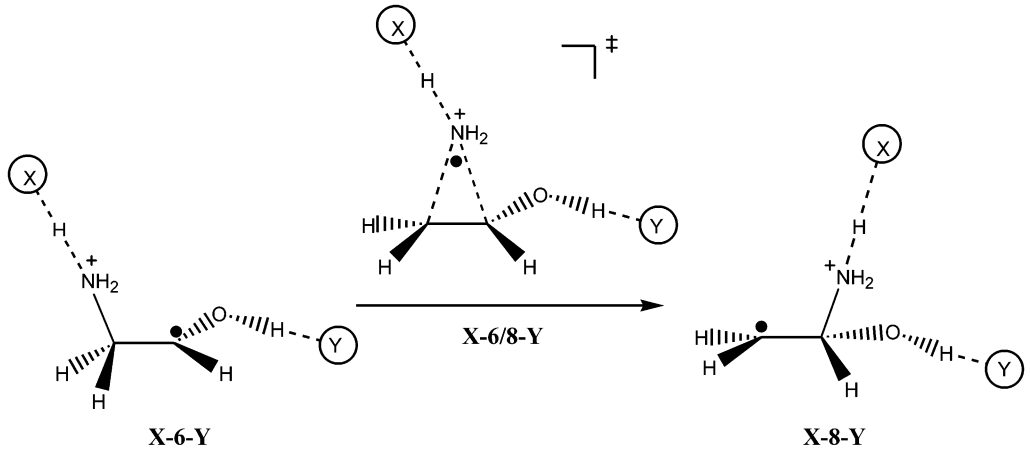


TABLE 7. Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel, 0\ K}$) and 298 K ($H_{rel, 298\ K}$) of the Stationary Points on the PES of 6 Interacting with Imidazole (Im) as a His Model System and Acetic Acid (Ac) as Asp/Glu Model System

	B3LYP/6-31G*		QCISD/cc-pVDZ//B3LYP/6-31G*	
	$H_{rel, 0\ K}$	$H_{rel, 298\ K}$	$H_{rel, 0\ K}$	$H_{rel, 298\ K}$
Im-6,[b]	0.0	0.0	0.0	0.0
Im-6/8[b]	19.1	19.3	27.2	27.4
Im-8[b]	7.8	7.8	5.3	5.3
Ac-6[c]	0.0	0.0	0.0	0.0
Ac-6/8[c]	15.5	15.8	24.2	24.2
Ac-8[c]	7.2	7.2	5.8	5.9

[a] For electronic energies, ZPEs, and enthalpies, see Table 7S in the Supporting Information. [b] **Im-6′** structure taken as a reference point. [c] **Ac-6′** structure taken as a reference point.

Thus, the migration pathway will be calculated employing acetic acid (rather than formic acid) as a model system for Asp/Glu and imidazole (rather than methanimine) as a model system for His.

(2) Acetic acid and imidazole—More reliable model systems for Asp/Glu and His.

For both model systems, the rearrangement commences from a conformer in which an intramolecular H-bond exists (see Figure 5); thus, the computed activation enthalpy corresponds to the final energy demand for the migration pathway. His is expected to catalytically interact with the substrate radical in the pH regime 6−9.5, while for Asp model system only interactions at pH < 4 were taken into account. When imidazole interacts with the NH_3 group of **6**, the activation enthalpy is quite high and is equal to 27.4 kcal/mol due to a higher degree of deprotonation in **Im-6′/8′** as compared to the analogous TS **Ac-6′/8′** (Figure 5). In the case of acetic acid, the activation enthalpy is somewhat lower at 24.2 kcal/mol (Table 7). While both models are structurally closer to the amino acids than the previously employed systems and, further, have proton affinities comparable to those of the amino acids,[54] the computed barriers, nevertheless, are too high to be acceptable for the enzymatic reaction. Even though partial protonation helps to lower the barrier for the rearrangement **6** → **8**, the amount gained is not sufficiently large to bring about acceleration of the enzymatic process. Clearly, in reality a more complex situation must prevail.

Synergistic Action of Two Catalytic Auxiliaries. One of the pathways by which the enzyme can reduce the activation enthalpy for the rearrangement process is the so-called push−pull mechanism proposed by Radom and co-workers in the case of diol dehydrase[17] and employed as well in the study of ethanolamine rearrangement catalyzed by ethanolamine ammonia lyase.[16] Since substrate **2** exhibits acidic (OH group) and basic (NH_2 group) features, both sites might interact with different amino acid residues of the enzyme as depicted in Scheme 5. Depending on the actual pH in the enzyme's active site, interaction is possible separately at the NH_2 or OH group or both sites of substrate **2** are simultaneously interacting with appropriate catalytic auxiliaries of the enzyme.

(1) Pull mechanism.

Since the interaction of a protonated HX^+ species with the NH_2 group has been investigated previously, we will first focus on the effect of a partial deprotonation of the OH group on the activation enthalpy. In our previous study on the aminoethanol radical rearrangement,[8] it was shown that for the nonprotonated form of substrate **2** a dissociation−association pathway is energetically least demanding; thus, this scenario was computed for the partial deprotonation as well (Scheme 6). We computed only the first reaction step (dissociation of the NH_2 group) for different deprotonating groups Y rather than computing the whole reaction profile that includes association as well. Clearly, if dissociation of the NH_2 group exceeds the activation enthalpy upper limit value, the pull mechanism alone will not play a role in the enzymatic catalysis.

First, the rather small OH^- ion was used as a deprotonating agent. Even though the activation enthalpy for the dissociation process (15.2 kcal/mol, Table 8) falls into the range acceptable for the enzymatic catalysis, bare OH^- as a model system for Asp/Glu is quite inappropriate. Thus, the interaction at the OH site in **2** was computed employing better model systems for the amino acids.

If the pH in the active site is higher than 4, Asp and Glu exist in their dissociated forms; thus, a carboxylate

(54) PA(Asp) = 217.2 kcal/mol and PA(Glu) = 218.2 kcal/mol vs PA(CH_3COOH) = 187.3 kcal/mol, PA(HCOOH) = 177.3 kcal/mol), PA(H_2O) = 165.0 kcal/mol; PA(His) vs PA (imidazole) = 225.3 kcal/mol, PA(CH_2NH) = 203.8 kcal/mol, PA(NH_3) = 204.0 kcal/mol. All data taken from ref 47.

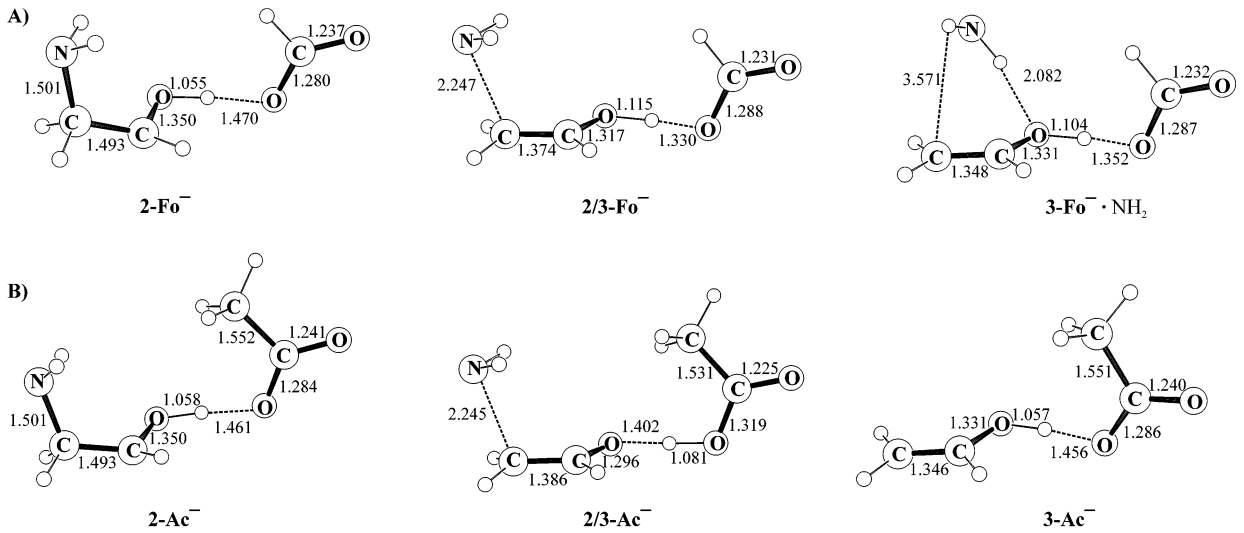


FIGURE 6. Pull mechanism; optimized geometries of stationary points relevant for the rearrangements of partially deprotonated aminoethanol radical **2** interacting with two Asp/Glu model systems: (A) formate and (B) acetate (bond lengths are given in Å; B3LYP results).

SCHEME 6. Pull Mechanism; the Dissociation-association Pathway

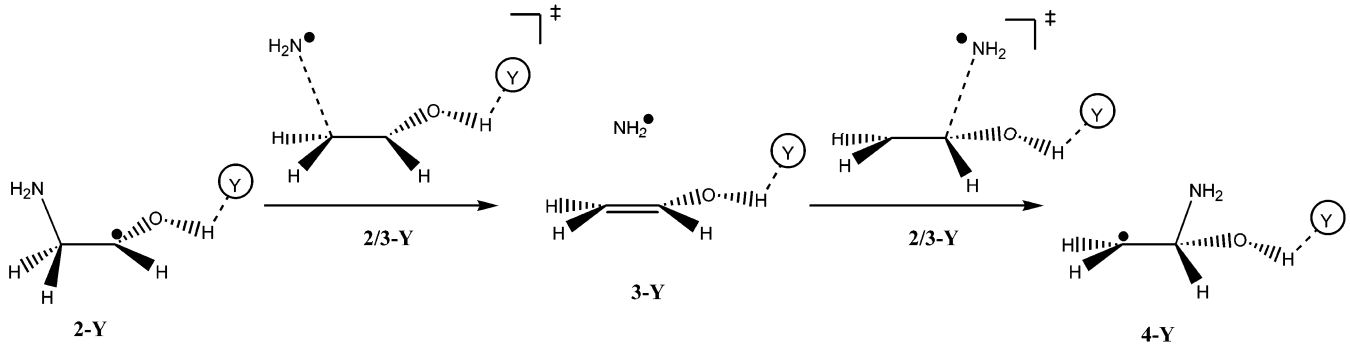


TABLE 8. Pull Mechanism; Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel,\ 0\ K}$) and 298 K ($H_{rel,\ 298\ K}$) of the Stationary Points on the PES of 2 Interacting with OH^- (O^-), HCO_2^- (Fo^-), and $CH_3CO_2^-$ (Ac^-) Which Serve as Dissociated Asp/Glu Model Systems

	B3LYP/6-31G*		QCISD/cc-pVDZ // B3LYP/6-31G*	
	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$
2O^{-b}	0.0	0.0	0.0	0.0
2/3O^{-b}	8.7	8.9	15.1	15.2
3O$^-\cdot NH_2{}^b$	7.7	8.6	12.8	13.7
2-Fo^{-c}	0.0	0.0	0.0	0.0
2/3-Fo^{-c}	14.7	14.9	19.5	19.7
3-Fo$^-\cdot NH_2{}^c$	11.3	12.4	8.6	9.7
2-Ac^{-d}	0.0	0.0	0.0	0.0
2/3-Ac^{-d}	14.6	14.8	20.0	20.2
3-Ac$^- + NH_2{}^d$	19.8	21.0	17.1	18.3

[a] For electronic energies, ZPEs, and enthalpies, see Table 8S in the Supporting Information. [b] **2O**$^-$ structure taken as a reference point. [c] **2-Fo**$^-$ structure taken as a reference point. [d] **2-Ac**$^-$ structure taken as a reference point.

TABLE 9. Pull Mechanism; Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel,\ 0\ K}$) and 298 K ($H_{rel,\ 298\ K}$) of the Stationary Points on the PES of 2 Interacting with CH_2NH (Mi) and Imidazole (Im) as His Model Systems

	B3LYP/6-31G*		QCISD/cc-pVDZ// B3LYP/6-31G*	
	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$
2-Mi^b	0.0	0.0	0.0	0.0
2/3-Mi^b	20.9	21.1	24.0	24.1
3-Mi $+ NH_2{}^b$	20.1	21.2	17.4	18.5
2-Im^c	0.0	0.0	0.0	0.0
2/3-Im^c	20.4	20.7	23.6	23.8
3-Im $+NH_2{}^c$	19.9	21.1	17.2	18.4

[a] For electronic energies, ZPEs, and enthalpies, see Table 9S in the Supporting Information. [b] **2-Mi** structure taken as a reference point. [c] **2-Im** structure taken as a reference point.

can *partially* deprotonate the OH group in **2**. The model systems employed in order to mimic these interactions were formate and acetate (see Figure 6). For the interaction of $HCOO^-$ with the OH group, the activation enthalpy for the dissociation of NH_2 is equal to 19.7 kcal/mol. When acetate as a model system for Asp/Glu was employed, the activation enthalpy is somewhat higher, being equal to 20.2 kcal/mol (Table 8).

His can act as a proton acceptor if the pH in the active site is higher than 6. When the smaller model system CH_2NH is used to partially deprotonate **2**, the computed activation enthalpy is 24.1 kcal/mol. In the case of a more realistic model system, i.e., imidazole, the activation enthalpy is somewhat lower, being equal to 23.8 kcal/mol (Table 9).

In general, partial deprotonation at the OH group of **2** acts catalytically as well, since in all investigated cases

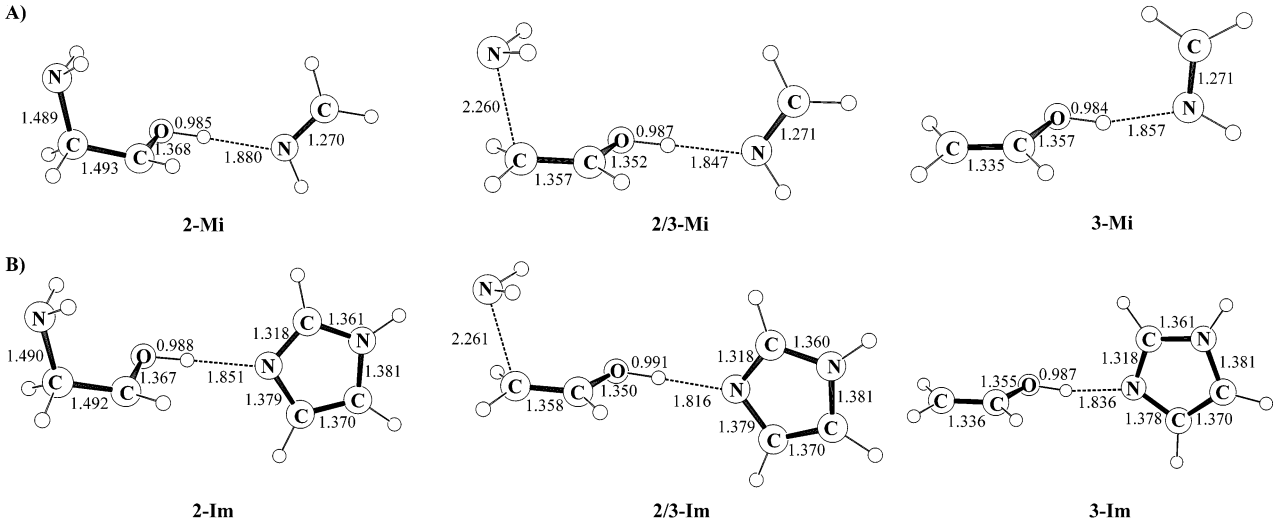


FIGURE 7. Pull mechanism; optimized geometries of stationary points relevant for the rearrangements of partially deprotonated aminoethanol radical **2** interacting with two His model systems: (A) CH_2NH and (B) imidazole (bond lengths are given in Å; B3LYP results).

the activation enthalpy obtained is lower than that for a nondeprotonated radical **2** (28.0 kcal/mol). However, the computed activation enthalpies for dissociation of the NH_2 group still exceed the upper limit determined from the experimental studies (ca. 15 kcal/mol); thus, the association of the NH_2 radical with **3-Y** (Scheme 6) was not deemed necessary to be investigated computationally. Clearly, a partial deprotonation at the OH group in **2**(pull mechanism) alone cannot sufficiently decrease the activation enthalpy. More likely, only when both interactions, i.e., partial deprotonation of the OH and partial protonation of the NH_2 group, occur simultaneously, the activation enthalpy might fall below 15 kcal/mol.

(2) Push−pull mechanism.

As already demonstrated by Radom and co-workers,[16] a push−pull mechanism (Scheme 5) might serve as the best model for the rearrangement of aminoethanol catalyzed by ethanolamine ammonia lyase. Even though the rearrangement barriers were calculated to be lower than the upper limit acceptable for the enzymatic reaction, one should be aware of the rather crude model systems (e.g., NH_3, H_2O) employed in the latter study.[16] Thus, we decided to calculate the activation enthalpies using some more realistic model systems for those amino acids that might act as catalytic auxiliaries. Clearly, these data are not only likely to result in more reliable activation enthalpies, but, as well, perhaps more importantly they might help to provide a realistic picture of possible interactions in the enzyme's active site.

Elucidation of the question which amino acid acts as a catalytic auxiliary at the hydroxyl and which at the amino group of **2** is related to the possible pH prevailing in the active site. Having in mind pK_a values of the potential amino acid candidates (Asp, Glu, His) and the pK_a of aminoethanol, it is clear that in the pH regime 6−9.5 a synergistic interaction can take place. In this pH range, Asp/Glu exist as carboxylate, His is nonprotonated, and the substrate **2** is protonated. In the section where aspects of partial protonation were discussed in detail, it was concluded that interaction of carboxylate with the NH_3 group in **6** substantially increases the activation enthalpy and is definitely not catalytic. Thus, only His is an acceptable candidate for the interaction with the NH_3 group of **6**. However, both His and Asp/Glu in this particular pH range might partially deprotonate the OH group of **6**. When discussing the pull mechanism, it was shown that the activation enthalpy is lower when model systems for Asp/Glu were employed in comparison with model systems for His. Thus, Asp/Glu were assumed to serve as better catalytic auxiliaries at the OH site in **6**.

To elucidate the push−pull mechanism in more detail, two different systems were employed. First, we used a set of smaller models for the above-mentioned amino acids (CH_2NH for His and $HCOO^-$ for Asp/Glu; Figure 8A). The computed activation enthalpy is 11.6 kcal/mol (Table 10), lower than the upper limit derived from experiment. When a more realistic model for the amino acids was used (imidazole for His and CH_3COO^- for Asp/Glu), the activation enthalpy is slightly higher, being equal to 13.7 kcal/mol. The analysis of the structural details (Figure 8) reveals that the low activation enthalpies are due to both reactant destabilization and transition structure stabilization. For example, in both structures **Mi-6-Fo**$^-$ and **Im-6-Ac**$^-$ the C(2)−N bonds are extremely prolonged when compared to the ones in **Mi-6$_1$** (Figure 2) or **Im-6** (Figure 5). At the same time, interaction of the carboxylate with the OH group of **6** results in a lengthening of the O−H bond; as a consequence, the system develops features of an ethanal structure (e.g., compare the C−C and C−O bond lengths given in Figure 8). The emerging radical center on C(2) is stabilized by delocalization through the developing ethanal structure. It is this subtle interplay of the two catalytic auxiliaries that makes the migration energetically less demanding than when compared to situations where only one of the catalytic auxiliaries is in action (e.g., **Im-6/8** or **2/3-Ac**$^-$). As a result, the activation enthalpy for the migration costs only 13.7 kcal/mol, which is lower than the upper limit determined from experiment (ca. 15 kcal/mol).[11,12]

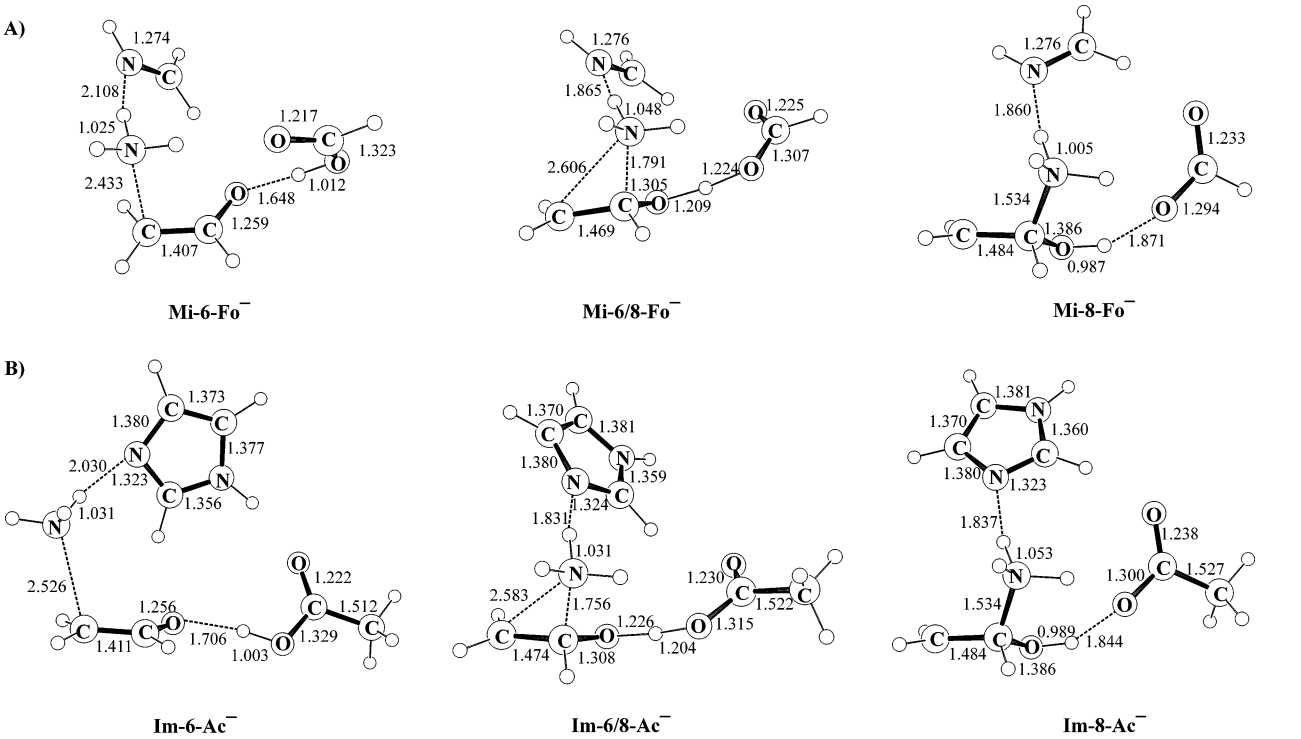


FIGURE 8. Push−pull mechanism; optimized geometries of stationary points relevant for the rearrangements of **6** interacting with His and Asp/Glu model systems: (A) CH_2NH and formate and (B) imidazole and acetate (bond lengths are given in Å; B3LYP results).

TABLE 10. Push−Pull Mechanism; Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel,\ 0\ K}$) and 298 K ($H_{rel,\ 298\ K}$) of the Stationary Points on the PES of 6 Interacting with Model Systems for His and Asp/Glu (CH_2NH with $HCOO^-$ and Imidazole with CH_3COO^-)

	B3LYP/6-31G*		QCISD/cc-pVDZ// B3LYP/6-31G*	
	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$	$H_{rel,\ 0\ K}$	$H_{rel,\ 298\ K}$
Mi-6-Fo^{-b}	0.0	0.0	0.0	0.0
Mi-6/8-Fo^{-b}	13.8	12.8	12.6	11.6
Mi-8-Fo^{-b}	9.3	8.3	5.3	4.3
Im-6-Ac^{-c}	0.0	0.0	0.0	0.0
Im-6/8-Ac^{-c}	16.9	15.7	14.9	13.7
Im-8-Ac^{-c}	13.0	11.9	8.7	7.6

[a] For electronic energies, ZPEs, and enthalpies, see Table 10S in the Supporting Information. [b] **Mi-6-Fo**$^-$ structure taken as a reference point. [c] **Im-6-Ac**$^-$ structure taken as a reference point.

Conclusion

The present computational study of the aminoethanol rearrangement, including different models for a partial substrate protonation, clearly discriminates between the two mechanisms (Scheme 2: direct NH_3 migration, **6** → **8,** vs NH_4^+ elimination, **6** → **10**), which were identified as the most likely routes in our previous study.[8] As a result, migration of the partially protonated amino group is shown to be energetically less demanding in all investigated cases in comparison to the elimination process.

However, even if realistic model systems for the amino acids His, Asp, and Glu, which can act as catalytic auxiliaries and partially protonate the substrate, are employed, the computed activation enthalpies exceed the upper limit value (ca. 15 kcal/mol) determined by kinetic studies as acceptable for enzymatic catalysis. For example, when imidazole is employed as a model system for His to interact with the NH_3 group of substrate **6**, the activation enthalpy for the migration process is 27.4 kcal/mol. If acetic acid is employed to mimic Asp or Glu interacting with NH_3 in **6**, the activation enthalpy is somewhat lower, being equal to 24.2 kcal/mol. Thus, partial protonation of the amino group in the substrate alone does not sufficiently reduce the activation enthalpy for this pathway to be feasible under enzymatic conditions. However, partial protonation of the amino group still acts catalytically since all computed activation enthalpies are lower than when compared to a rearrangement of the nonprotonated substrate radical **2** (28 kcal/mol).

In the case of a partial deprotonation of the substrate **2** at the OH group, the rearrangement mechanism consists of a dissociation of the NH_2 radical from C(2) and its association at the C(1) atom. For all investigated proton acceptors (OH^-, HCO^-, CH_3COO^-, CH_2NH, imidazole), the activation enthalpy for the dissociation step exceeds the limit value. Typical data are 20.2 kcal/mol for acetate and 23.8 kcal/mol for imidazole interacting with the OH group of **2**; thus, Asp or Glu present better candidates than His to pull a proton from the OH group of the substrate. As in the case of partial protonation of the NH_2 group, the partial deprotonation of the OH group acts catalytically in that it reduces the activation enthalpy, though not sufficiently.

Obviously, a synergistic action (Scheme 5) of two catalytic auxiliaries in the enzyme's active site is necessary to result in a sufficient reduction of the activation enthalpy. Elucidation of the question which amino acid acts as a catalytic auxiliary at the OH (partial deprotonation) and which at the NH_2 group (partial protonation)

of **2** depends on the possible pH in the active site. Only in a pH regime 6−9.5 the synergistic interaction can take place; in this pH range Asp/Glu exist as carboxylates and His is nonprotonated while the substrate is still protonated. Thus, His is a better candidate for an interaction with the NH_3 group in substrate **6**. However, in this particular pH range both His and Asp/Glu might partially deprotonate the OH group in **6**. Since it was shown for the pull mechanism (Scheme 6) that the activation enthalpy is lowest when model systems for Asp/Glu were employed in comparison with model systems for His, in the deprotonation step either Asp or Glu are predicted to be involved.

Details of the push−pull mechanism were calculated employing two different systems (Figure 8). When the smaller models for the catalytic auxiliaries His and Asp/Glu (i.e., CH_2NH and $HCOO^-$) were used, the computed activation enthalpy is 11.6 kcal/mol lower than the upper limit determined from experiment. For more realistic models (i.e., imidazole and CH_3COO^-), the activation enthalpy is slightly higher (13.7 kcal/mol) but still *lower* than the upper limit value determined experimentally (ca. 15 kcal/mol). Further, this activation enthalpy is *lower* than the barrier associated with hydrogen abstraction from the 5′-deoxyadenosine by the product radical; this process was shown to be the rate-determining step in the overall reaction sequence. In addition, the synergistic interaction of His and Asp/Glu is operative only in the pH regime of 6−9.5; this pH range is common in many biologically active systems.[55]

Finally, the computational data reported here do not only provide reliable activation enthalpies, more importantly, they produce quite a realistic picture of possible interactions in the enzyme's active site. These findings may prove helpful in the ongoing experimental attempts to structurally characterize ethanolamine ammonia lyase.[56]

Acknowledgment. We thank Professor Leo Radom for providing us with a preprint of a manuscript dealing with a related topic and an anonymous reviewer for insightful comments. Financial support by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged. We thank the Konrad-Zuse Zentrum for the generous allocation of computer time, and M.S. is grateful to the Ernst Schering Research Foundation for a fellowship. This paper is dedicated to Professor Rudolf Wiechert on the occasion of his 75th birthday.

Supporting Information Available: Tables of electronic energies, zero-point energies, and enthalpies of the stationary points on the potential energy surface as well as the appendix on the structure labeling code and Cartesian coordinates of the transition structures. This material is available free of charge via the Internet at http://pubs.cas.org.

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(55) For example, the optimal pH for catalytic activity of related glutamate mutase falls into the range 7.5−8; see ref 28.

(56) For recent review articles on this and related topics, see: (a) Banerjee, R. *Chem. Rev.* **2003**, *103*, 2083; (b) Toraya, T. *Chem. Rev.* **2003**, *103*, 2095; (c) Hind, F.; Siegbahn, P. E. M. *Chem. Rev.* **2003**, *103*, 2421.