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Computational Exploration of Rearrangements Related to the Vitamin B_{12}-Dependent Ethanolamine Ammonia Lyase Catalyzed Transformation

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Abstract: DFT (B3LYP/6-31G*) and ab initio molecular orbital theory (QCISD/cc-pVDZ) are used to investigate several possible mechanisms involving free radical intermediates as well as their protonated forms for processes related to the coenzyme B_{12}-dependent rearrangement catalyzed by ethanolamine ammonia lyase. Two major types of rearrangements are discussed in detail, intramolecular migration and dissociation of the amine/ammonia groups, for both of which several scenarios are considered. According to the calculations, the complete dissociation of the migrating group and its subsequent association constitute an unlikely route for both the protonated and the unprotonated reactant because of the high-energy barriers (more than 23 kcal/mol) involved in these steps. Direct migration of the protonated amine group is far more favorable (10.4 kcal/mol) and therefore presents the most likely candidate for the actual enzymatic reaction. The calculations further imply that the direct loss of an ammonium cation (10.6 kcal/mol) represents a feasible pathway as well. Comparing the rearrangements for the aminoethanol radical and its protonated counterpart, in line with previous findings reported by Golding, Radom, and co-workers, we find that the migration of a protonated group is in general associated with lower energy barriers, suggesting that the actual enzyme substrate quite likely corresponds to (partially) protonated aminoethanol. As the extent of the substrate protonation/deprotonation by the active site of the enzyme may vary, the actual energy barriers are expected to range between the values calculated for the two extreme cases of a substrate, that is, the aminoethanol radical **2** and its fully protonated form **6**.

Introduction

The vitamin B_{12} coenzyme-dependent enzymes catalyze homolytic cleavage of the C−H bond,[1,2] and it is generally believed that radical intermediates are involved in the subsequent 1,2-migration of hydrogen, alkyl, carbonyl, hydroxyl, or amide groups.[1−3] Ethanolamine ammonia lyase[4] from bacteria metabolizes the substrate aminoethanol,[5] **1**, to ethanal, **11**, and ammonia (Scheme 1).[6] Even though the best substrate for this reaction is **1**, the enzyme can utilize a number of other 2-amino alcohols as well. The ethanolamine ammonia lyase requires the presence of the vitamin B_{12} coenzyme for its catalytic activity. A central part of the catalytic role of vitamin B_{12} stems from the relative weakness of its cobalt−carbon bond with a dissociation energy less than 30 kcal/mol.[7] The energy required for that homolysis is delivered by a conformational change in the protein induced by binding of the substrate, and homolysis of the Co−C bond is accelerated up to a factor of 10^{12} in the presence of an enzyme.[8] 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, and it is the latter radical that has been proposed to abstract a hydrogen atom from aminoethanol (Scheme 1: **1** → **2**).[9] There are some indications that the protein-associated radical could participate in that step as well.[10] As for the initially formed intermediates involved in the subsequent rearrangement of **2**, ambiguities still exist. Two basic pathways have been proposed (Scheme 1):[11] an amine-migration pathway (**2** → **4**) and an amine-dissociation pathway (**2** → **10**). Both routes yield eventually the same final products, that is,

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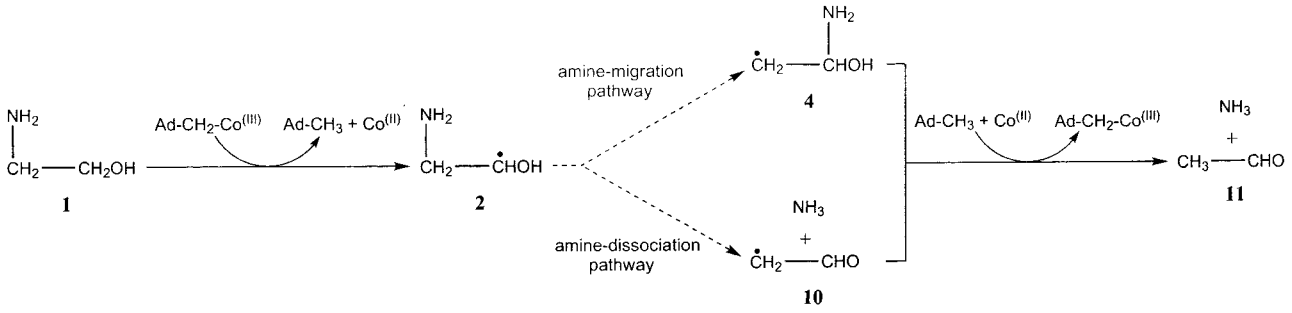
Scheme 1. Possible Rearrangement Paths in the Deamination of Aminoethanol, **1**, by Ethanolamine Ammonia Lyase (See Text and Subsequent Schemes for the Structure Labeling)



ethanal, **11**, and ammonia; **11** is formed by reabstraction of a hydrogen atom from the 5′-deoxyadenosine, thus regenerating the adenosyl-Cob(III)alamin and closing the catalytic cycle. As far as the rearrangement of **2** is concerned, despite elegant EPR experiments[12] and circumstantial evidence, the detailed mechanistic picture of this and related B_{12} catalyzed transformations is the least understood aspect in the bound free-radical hypothesis.

The mechanistic dichotomy depicted in Scheme 1 seems to exist for other systems as well, and under particular conditions radical-mediated rearrangements were shown to be facile as compared to those with closed-shell species. Often, the energy barriers for a carbon−heteroatom bond cleavage, caused by a neighboring radical center, decrease to one-half of the value for rearrangement barriers proceeding through closed-shell intermediates.[13] As far as gas-phase studies of aminoalkanes are concerned, for the protonated ethylamine it was shown that the ammonium ion elimination is the energetically preferred path.[14]

Recently, quite a few quantum-mechanical studies have been reported on B_{12}-mediated rearrangements, and noteworthy are the following cases: methylmalonyl-CoA,[15] 1,2-diols,[16−18] 2-methyleneglutarate,[19] and the aminomutase catalyzed 1,2-amino shifts in amino acids.[20] The 1,2-amino migrations catalyzed by aminomutases differ from the formally related 1,2-amino shift catalyzed by ethanolamine ammonia lyase because of the fact that the former depends on the interaction of the substrate (amino acid) with another vitamin (i.e., vitamin B_6), while the latter rearrangement proceeds by cooperative action of the enzyme and vitamin B_{12} only. The intriguing concept of a partially protonated migrating group suggested by Smith, Golding, and Radom[15,16] seems to play an important role in the enzyme activity. Because of partial protonation, the energy barriers of the corresponding rearrangements are lowered to the extent that they get close to the energetics pertinent to enzyme catalysis.

The ethanolamine ammonia lyase dependent rearrangement of aminoethanol has not been the subject of theoretical studies so far. In the present computational investigation, several mechanistic scenarios will be addressed including the concept of partial protonation of the substrate. The latter mode of operation is supported by the finding that in several B_{12}-dependent enzymes the amino acid sequence of the enzyme active site contains Asp and His residues,[21] which might serve as proton donors. In the case of methylmalonyl-CoA mutase catalyzed rearrangement, it was concluded that His plays a role of a proton donor.[22] From the aminoethanol acidity ($pK_a = 9.45$ for the conjugate acid of **1**), one can expect partial, if not complete, protonation of the aminoethanol substrate embedded in the enzyme's active site.

Computational Methods

All calculations were performed with the Gaussian 98 suite of programs[23] 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 used.[24,25] It should be mentioned that in related studies[26] B3LYP calculations have provided good agreement with the experimental data as well as with the data obtained with the high-level theoretical methods.

Geometry optimizations were performed with Pople's polarized double-ζ 6-31G* basis set.[27] To characterize the optimized structures, frequency analysis has been performed at the same level of theory. Minima were characterized by the absence of imaginary vibrational

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frequencies, while transition structures exhibited one imaginary frequency. Computations of reaction pathways (IRC, relax PES scans) were carried out at the same level of theory.

Because the B3LYP method occasionally performs quite unsatisfactory in the case of the reaction enthalpy evaluations,[28] geometry reoptimizations were performed at the QCISD level of theory using Dunning's correlation-consistent double-ζ basis set cc-pVDZ[29] to obtain more accurate energetic profiles of the reactions in question, as well as the geometries of the stationary points. The CBS-RAD (QCISD, B3LYP) method[30] has not been applied because the computational cost would be even higher, and the energetic picture of the overall rearrangement pathways would not change dramatically. A uniformed scaling factor of 0.9806 was used for the zero-point energy (ZPE) corrections obtained at the B3LYP level of theory.[31] The relative energies in the text (given in kcal/mol) correspond to the enthalpies at 298 K obtained at the QCISD level of theory, unless specified otherwise. The electronic energies, ZPEs, and the enthalpies of stationary points can be found in the Supporting Information.

Inclusion of solvent, for example, water molecules, in the calculations is not indicated on the ground that hydrogen exchange has not been observed in vitamin B_{12}-dependent rearrangements.[2c] We also refrain from including any specific interaction of the substrate **1** with amino acids. While the amino acid sequence of the enzyme has been determined,[32] the X-ray structure of the enzyme is not yet known. Consequently, no information is available on how the amino acids relevant for a protonation of **1** are positioned in the active site. Therefore, for the time being, a comprehensive computational analysis of the isolated substrate seems to be the most promising way of providing insight into the energetically most feasible mechanistic scenarios operating in the actual rearrangement of **2** (Scheme 1).

Results and Discussion

In the computations, various rearrangement possibilities of the neutral aminoethanol radical **2**, and its N-protonated counterpart **6** (Schemes 2, 4), were considered. The structure labels in Schemes 2 and 4 comprise the whole conformational space to which a structure in question belongs, while in the text, when discussing the mechanisms, a particular computationally characterized conformer is addressed. When more than one conformer was obtained during the calculations, the subscript of the label points to a specific conformer. The relative enthalpies at 298 K of the stationary points are presented in Tables 1 and 2, and the optimized geometries of minima are presented in Figure 1; geometrical parameters of transition structures can be found in Figure 2. Comparing the radical geometries obtained at both levels of theory (Figures 1 and 2), we found that the most pronounced bond-length differences exist in the transition structures. The QCISD method is believed to provide a more accurate description of the actual geometries.[33] As expected, for the closed-shell species, both methods give nearly identical results (see Figure 1). As to the energetics, all B3LYP barriers are lower than the ones obtained at the QCISD level of theory; this confirms the well-known B3LYP underestimation of transition barriers for radical-mediated rearrangements.[34,35]

The conformational analysis of aminoethanol **1** was the subject of several theoretical studies so far.[36,37] Out of the total of 27 conformers, a pronounced hydrogen bond bridging the two functional groups can be found in several of them. The most stable conformer,[38] **1** (Figure 1), exhibits the strongest stabilization because of a hydrogen bond between the nitrogen atom and the hydrogen from the OH group ($d_{NH} = 2.179$ Å).

Concerning the radical **2**, several conformers were considered for the reaction pathway calculations. The hydrogen bond is again an important factor for the structure stabilization. In the $\mathbf{2_1}$ conformer (Figure 1), a H-bond exists between a hydrogen atom from the NH_2 group and oxygen ($d_{HO} = 2.767$ Å); this conformer corresponds to the third stable structure of aminoethanol.[37] The conformer $\mathbf{2_2}$ is structurally related to the global minimum **1**. In $\mathbf{2_2}$ the N−H distance of 2.074 Å is even shorter than that in **1** (2.179 Å); thus, the H-bond is even stronger in the radical. The third conformer of the aminoethanol radical, that is, $\mathbf{2_3}$ (Figure 1), does not exhibit any stabilization through a H-bond; it is energetically less stable than the two cases mentioned above (by 5.2 kcal/mol relative to $\mathbf{2_2}$). While the most stable conformer $\mathbf{2_2}$ may serve as a good candidate for the direct loss of ammonia (see further in text), this conformer is not likely to play a role in the amino-group migration toward the electron-deficient carbon atom. For this rearrangement, conformers $\mathbf{2_1}$ and $\mathbf{2_3}$ are better candidates.

Intramolecular Migration of NH_x ($x = 2, 3$). Dissociation/Association Mechanism of Aminoethanol Radical 2 and Its Protonated Form 6 (Scheme 2A and D). The activation enthalpy for the dissociation of the aminoethanol radical $\mathbf{2_1}$ into ethenol, **3**, and NH_2 equals 24.1 kcal/mol; this barrier is somewhat higher than the one for the C−N bond cleavage of the aminoethyl radical.[20] IRC calculations from the $\mathbf{2_1/3}$ transition structure in the direction of the reactant resulted in the $\mathbf{2_1}$ conformer. The optimization of the IRC structure in the product direction (**3** and NH_2) did not indicate the existence of a complex between the two species. Instead, the structure breaks into two separate species; thus, convergence could not be achieved. Therefore, the combined energy of the products was obtained by calculating the energies of the separately optimized geometries of **3** and the NH_2 radical. A further possibility of the cleavage of $\mathbf{2_1}$ has been considered as well; however, even if permitted extensive solvation, the formation of a radical-cation **7** and an NH_2 anion can be excluded because the energy sum relative to $\mathbf{2_1}$ equals 248.7 kcal/mol. With regard to the final rearrangement product, $\mathbf{4_1}$, the transition structure $\mathbf{3/4_1}$ has been located. In analogy with the $\mathbf{2_1/3}$ transition structure, the optimization of the IRC structure in a direction of the reactant could not converge. Assuming that the reaction proceeds indeed via **3** and the $NH_2^{\bullet}$ radical, we found that the energy required to overcome the $\mathbf{3/4_1}$ transition state barrier equals 25.6 kcal/mol relative to $\mathbf{2_1}$. Obviously, path 2A is energetically too demanding to account for the enzyme-mediated rearrangement of **2**, as the energy required for the rate-determining step below 20 kcal/mol is considered necessary for an enzymatic reaction.[17,26]

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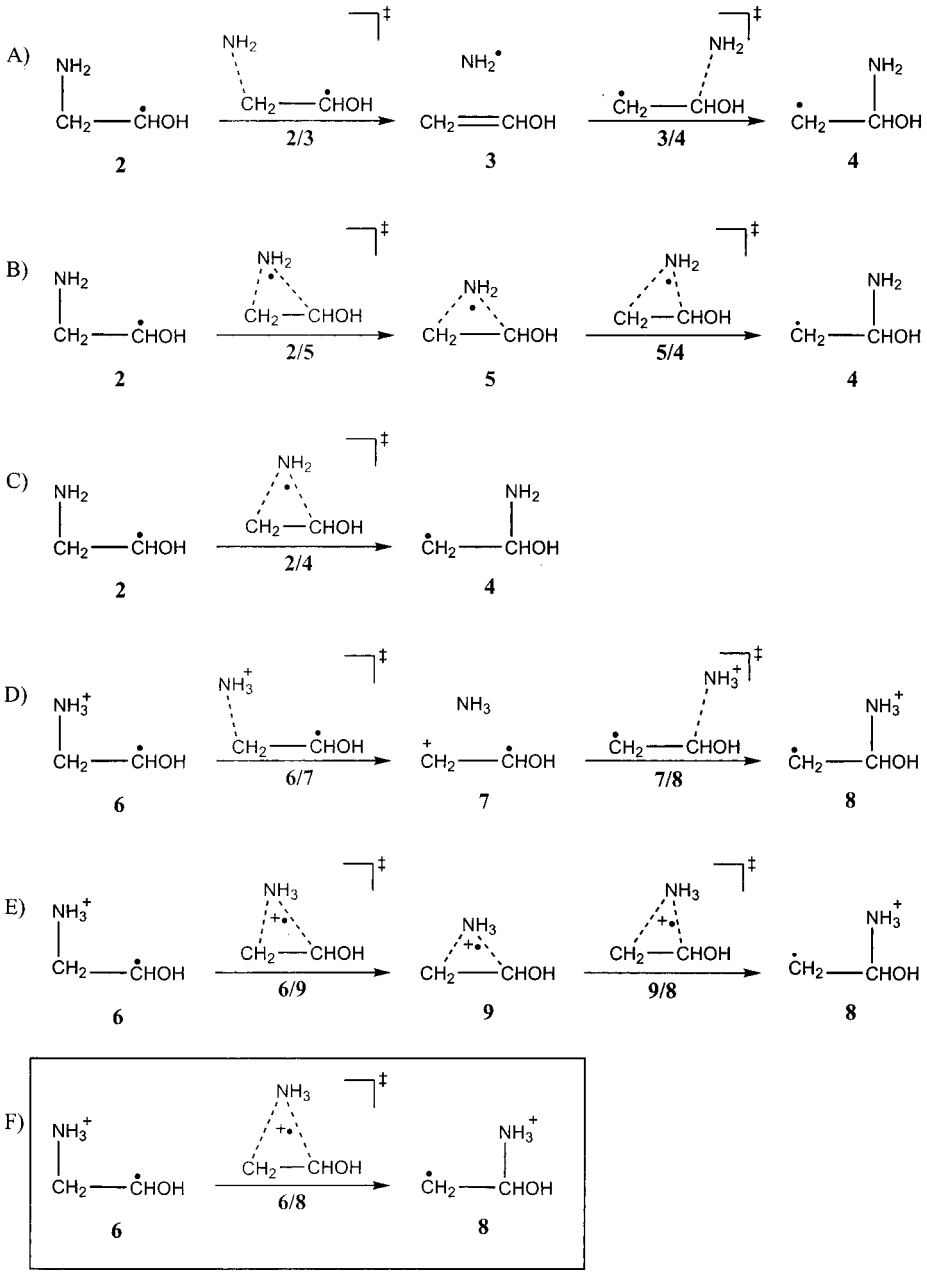
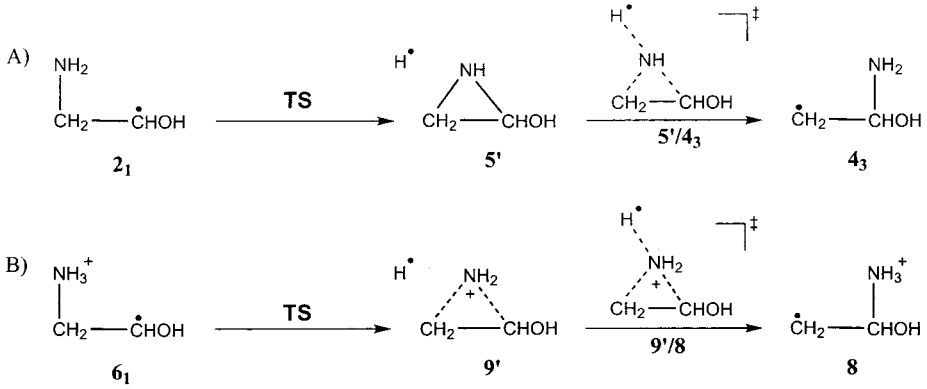
Scheme 2. NH_x ($x = 2, 3$) Migration Pathway of **2** and **6** Resulting in the Formation of 1-Aminoethanol Radical, **4**, and the N-Protonated Counterpart, **8** (Encircled Rearrangement **6** → **8** Corresponds to the Energetically Preferred Path)

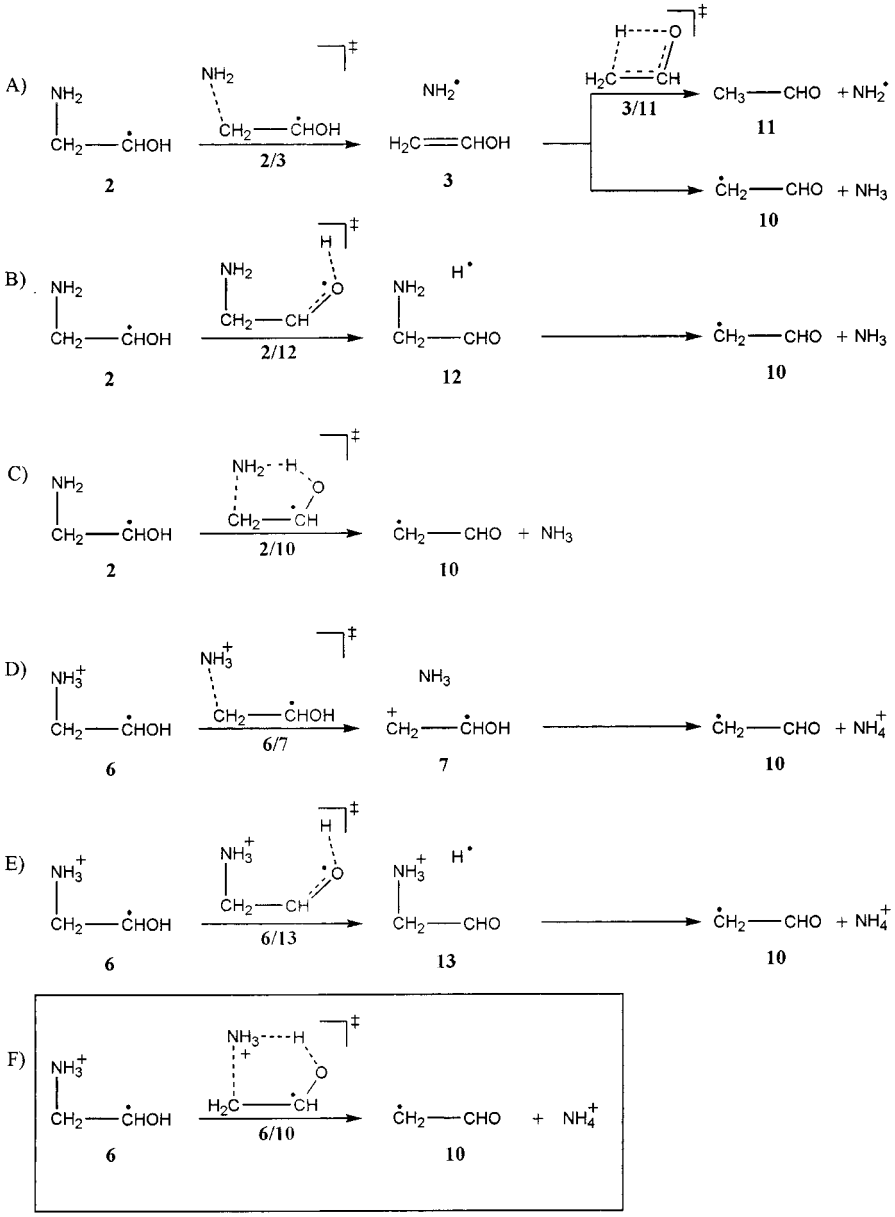


Scheme 3. NH_x ($x = 2, 3$) Migration Accompanied by Hydrogen Atom Dissociation/Association



For the related reactions of the protonated radical **6** (Scheme 2D), the transition structures **6/7** and **7/8** were not located. Nevertheless, the combined energy of the intermediate enol radical cation **7** and ammonia (Table 2) is too high to make

Scheme 4. Dissociation Pathways for the Formation of the 2-Ethanal Radical, **10** (Encircled Rearrangement **6** → **10** Corresponds to the Energetically Preferred Path)



this route a likely pathway in the rearrangement of the protonated radical $\mathbf{6_1}$. While the energy sum of the products **7** and NH_3 of the first rearrangement step equals already 29.2 kcal/mol, relative to $\mathbf{6_1}$, this situation gets worse energetically if ethenol **3** and the radical cation $NH_3^{+\bullet}$ are considered as a possible pair of products; the combined energy of **3** and $NH_3^{+\bullet}$, relative to $\mathbf{6_1}$, amounts to 47.0 kcal/mol. Clearly, this pathway is energetically not accessible for a fast enzymatic reaction, even if the enzyme might stabilize substantially the transition states of the dissociation/association pathways 2A and D.

Sequential Intramolecular Isomerizations of 2 and 6 (Scheme 2B and E). All attempts to locate the cyclic structure **5** as a minimum on the PES failed. During geometry optimization, one of the hydrogen atoms dissociates from the NH_2 group, forming the closed-shell cyclic structure **5′** (see Scheme 3A). The final product $\mathbf{4_3}$ (a conformer of **4** obtained with the IRC calculation from the corresponding TS $\mathbf{5'/4_3}$) can be formed through the transition structure $\mathbf{5'/4_3}$, which is associated with re-addition of the hydrogen atom (the activation enthalpy relative to $\mathbf{2_1}$ equals 58.1 kcal/mol). Again, and in line with findings on related processes,[26] the energies of all species characteristic for the sequence depicted in Scheme 3A are much too high to play a role in an enzymatic reaction.

A nonclassical structure proposed by George et al.[17] in the related isomerization of 1,2-ethanediol, where a hydrogen atom from an OH group bridges the C−C bond, could not be found as a stationary point of any kind for the isomerization of **2**. Similarly, in that study,[17] a classical structure where oxygen, rather than NH_2, is the bridging group could not be located either.

Some of the problems encountered in the reaction pathway calculation, discussed in the previous section, were faced in the case of the protonated radical **6** as well. Instead of locating the cyclic protonated radical **9** (Scheme 2E), we obtained the closed-shell cyclic cation **9′**, which was formed upon geometry optimization of **9**. The transition structure **9′/8** involved in the

Table 1. Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel,0K}$) and 298 K ($H_{rel,298K}$) of the Stationary Points on the Aminoethanol Radical PES

	B3LYP/6-31G*		QCISD/cc-pVDZ	
	$H_{rel,0K}$	$H_{rel,298K}$	$H_{rel,0K}$	$H_{rel,298K}$
2_1	0.0	0.0	0.0	0.0
2_2	−4.1	−4.4	−4.1	−4.4
2_3	1.0	1.1	0.8	0.8
4_1	3.7	3.6	−0.8	−0.9
4_2	3.2	3.6	1.5	1.9
4_3	−0.2	−0.1	−1.6	−1.6
2_1/3	20.4	20.6	23.9	24.1
3/4_1	23.0	23.2	25.5	25.6
5′/4_3	54.7	54.4	58.6	58.1
2_3/4_2	72.5	72.4	79.1	79.0
2_2/10	16.9	16.4	25.7	25.2
2_1/12	28.0	28.2	33.7	33.9
5′ + H•	44.6	45.4	43.2	43.9
3 + NH_2•	18.1	19.4	15.6	16.8
7 + NH_2^-	241.4	242.8	247.5	248.7
10 + NH_3	−6.8	−5.5	−5.4	−4.3
12 + H•	21.0	22.0	19.4	20.4

[a] For electronic energies, ZPEs, and enthalpies, see Table 1S in the Supporting Information.

Table 2. Relative Enthalpies[a] (in kcal/mol) at 0 K ($H_{rel,0K}$) and 298 K ($H_{rel,298K}$) of the Stationary Points on the Protonated Aminoethanol Radical PES

	B3LYP/6-31G*		QCISD/cc-pVDZ	
	$H_{rel,0K}$	$H_{rel,298K}$	$H_{rel,0K}$	$H_{rel,298K}$
6_1	0.0	0.0	0.0	0.0
6_2	0.4	0.6	1.5	1.6
8	2.9	3.0	−0.3	−0.1
6_1/8	4.7	4.9	10.2	10.4
6_2/10	8.8	9.1	12.0	12.2
9′/8	69.1	68.7	73.3	72.7
10 · NH_4^+	−17.9	−17.1	−16.7	−16.0
6_1/13	33.8	33.5	38.4	38.1
9′ + H•	48.9	49.5	46.5	47.0
3 + NH_3^{+}•	52.2	53.3	46.0	47.0
7 + NH_3	29.3	30.4	28.2	29.2
10 + NH_4^+	6.7	7.7	5.4	6.4
13 + H•	27.7	28.4	24.3	25.0

[a] For electronic energies, ZPEs, and enthalpies, see Table 2S in the Supporting Information.

formation of **8** was found to lie 72.7 kcal/mol above **6_1** (Scheme 3B), thus discarding this route in the isomerization of **6**.

One-Step Migration of NH_2/NH_3 in 2 and 6 (Scheme 2C and F). All stationary points of interest have been located on the respective PESs. The IRC calculations performed from the transition structure **2_3/4_2** lead to the conformers **2_3** and **4_2**. The barrier for a direct migration of a NH_2 group amounts to 78.2 kcal/mol, and this pathway is therefore not expected to play a role in the enzymatic reaction.

In contrast to the radical **2**, the activation enthalpy for the intramolecular transfer of the NH_3 group starting from **6_1** equals only 10.4 kcal/mol, thus clearly falling into the energy range typical for enzyme-catalyzed reactions.[26] The analogous barrier in the case of the ammonium-ethyl radical was calculated to be 25.0 kcal/mol for the investigation of a 1,2-amino shift catalyzed by aminomutases.[20] Obviously, the presence of the OH group at the radical terminus dramatically influences the transition state in such a way that the rearrangement is feasible even without the action of an additional cofactor, for example, B_6, as in the case of the 1,2-amino shift catalyzed by aminomutases.[20] Apparently, the spin delocalization through an additional heteroatom makes the migration more feasible.

The origin for the huge difference in the activation enthalpies between the migration of a neutral versus a charged group is most likely because of the bond redistribution in the two transition structures. In the case of a protonated group migration, TS **6_1/8** corresponds closely to **7** and NH_3, which can be inferred from the C−N bond lengths (see Figures 1 and 2). The enol radical cation **7** is more stable than its keto counterpart because of a better spin delocalization in the C−C−O backbone;[39] such a spin delocalization stabilizes the transition structure as well. The slightly preferred interaction of NH_3 with the C(1) center, on which the positive charge emerges, is reflected in a shorter N−C(1) distance with respect to the second C(2)−N bond. As further suggested by the calculations, the spin density at C(2) exceeds that of C(1). In contrast, in the **2_3/4_2** transition structure, a three-center three-electron bond is present through a delocalization of electrons between nitrogen and two carbon atoms. Any significant delocalization of the spin density between C(1) and C(2), which would correspond to a partial CC double-bond formation, can be neglected because of the fact that the C−C bond in **2_3/4_2** is actually elongated if compared to **2_3** (see Figures 1 and 2). The two C−N bonds of **2_3/4_2** are much shorter than those in the related **6_1/8** transition structure, showing a strong interaction between the NH_2 group and the C_2 backbone. The formation of such a strained structure with an extra electron in a high-lying orbital is obviously energetically more demanding than the corresponding **6_1/8** transition structure.

We note in passing that recent gas-phase experiments showed that migration of an NH_3 group is a rapid and common isomerization process in the case of protonated β-aminoalkyl radicals.[40]

Dissociation Pathways of 2 and 6. Concerning the role of stepwise NH_x ($x = 2, 3$) dissociation reactions, two mechanisms were considered, which differ in the details of the initial step. The reaction can commence either by the cleavage of the C−N bond (elimination of NH_2 or NH_3) or via hydrogen atom elimination from the OH group.

Elimination of the NH_x ($x = 2, 3$) Group as the Initial Step (Scheme 4A and D). These two initial steps have already been discussed in the context of dissociation/association mechanisms (see Scheme 2A and D) and therefore need not to be analyzed any further.

From the enol **3**, ethanal, **11**, could be formed directly through keto−enol tautomerization. The corresponding transition structure **3/11** for the unimolecular 1,3-H migration[41] lies 56.6 kcal/mol above **3**; thus, this symmetry-forbidden route can be discarded because of its high-energy demand. Besides, for that mechanistic proposal, the NH_2 radical formed in the step **2_1** → **3** should then be capable of abstracting a hydrogen atom from 5′-deoxyadenosine; however, according to EPR experiments, a hydrogen atom is abstracted by either 1-aminoethanol **8** or ethanal **10** radicals.[42] Formation of **10** through the homolytic bond cleavages of the O−H bond in **3** or a C−H in **11** is

(39) (a) Smit, B. J.; Nguyen, M. T.; Bouma, W. J.; Radom, L. *J. Am. Chem. Soc.* **1991**, *113*, 6452. (b) Turecek, F.; Cramer, C. J. *J. Am. Chem. Soc.* **1995**, *117*, 12243. (c) Rodríguez-Santiago, L.; Vendrell, O.; Tejero, I.; Sodupe, M.; Bertran, J. *Chem. Phys. Lett.* **2001**, *334*, 112.

(40) Hammerum, S.; Petersen, A. C.; Sølling, T. I.; Vulpius, T.; Zappey, H. *J. Chem. Soc., Perkin Trans. 2* **1997**, 391.

(41) Because an isotope exchange has not been observed between the substrate and the solvent in the vitamin B_{12}-dependent rearrangements, the variant of an intermolecular keto−enol tautomerization, catalyzed by an acid or a base, need not to be considered.

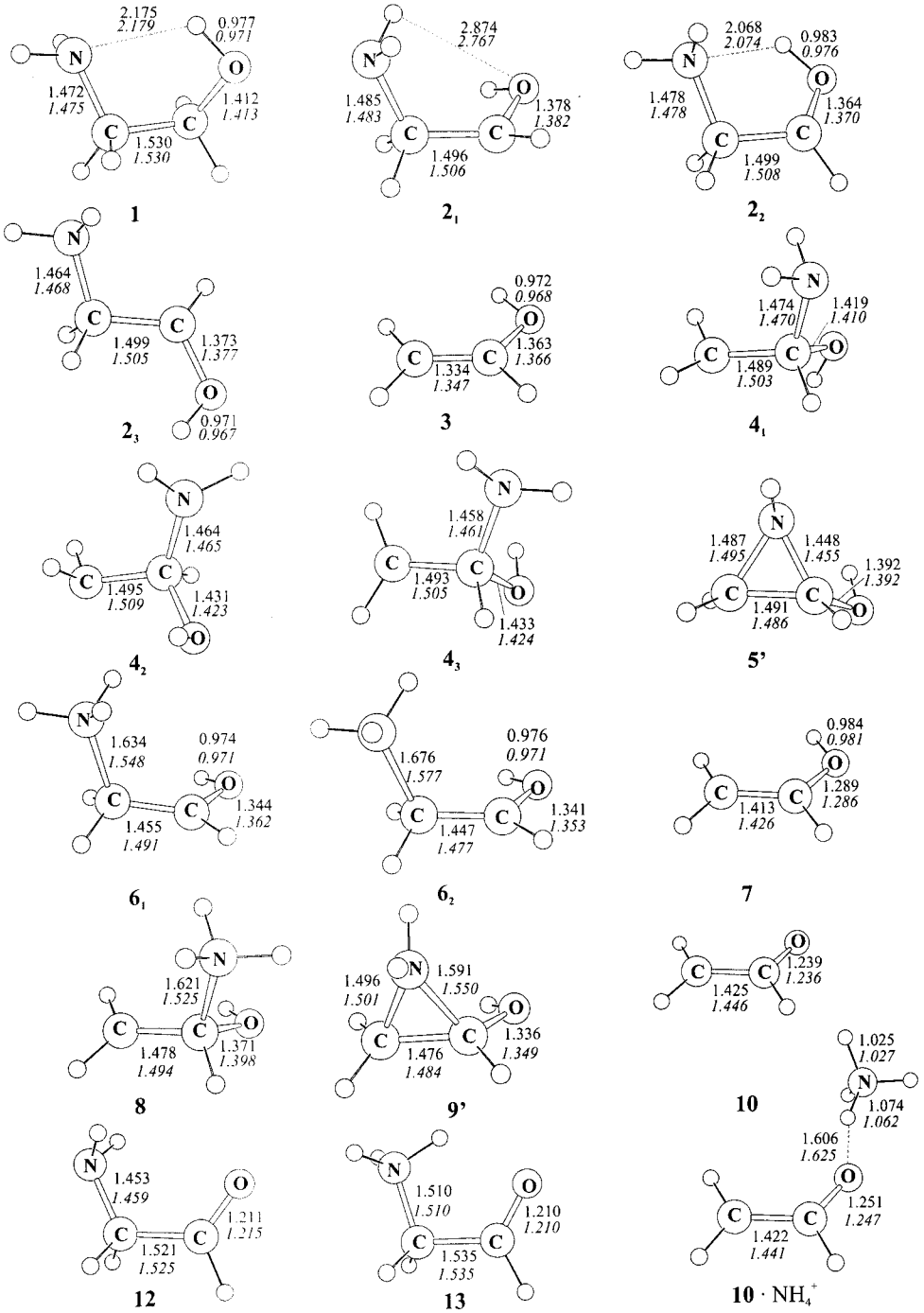


Figure 1. Optimized geometries of minima relevant for the rearrangements of **2** and **6**; bond lengths are given in Å (B3LYP results in roman and QCISD in italics).

compatible with the expected high-energy demands, that is, 76.9 and 90.5 kcal/mol, respectively. If one assumes that the **3** → **10** transformation is supported by the NH_2 radical formed in the previous step, then the reaction becomes exothermic (−21.1 kcal/mol relative to **3** + $NH_2^{\bullet}$). Even though the overall reaction energetics starting from $\mathbf{2_1}$ turn out to be almost thermoneutral (3.0 kcal/mol; taking into account the energy-demanding first step and energy-releasing second one), the stepwise elimination mechanism starting with an amino-group elimination is highly unlikely as the initial step $\mathbf{2_1} \rightarrow \mathbf{3}$ is energetically too demanding.

If a homolytic O−H bond cleavage was to take place in structure **7** (Scheme 4D), a hydrogen radical and the acetyl cation[43,44] would be formed, and the combined energy relative to **7** equals 21.1 kcal/mol. A heterolytic O−H bond cleavage of **7** to produce **10** and a proton would be even more demanding

(42) According to the IUPAC rules, more appropriate names for these radicals are 2-amino-2-oxoethyl and 2-oxoethyl radicals.

(43) The *n*-CH_2CHO^+ cation does not exist as a minimum on the PES. As already shown (see ref 44), the global minimum on the $C_2H_3O^+$ PES corresponds to the acetyl cation (CH_3CO^+); thus, its formation was assumed in our investigation as well. At the B3LYP/6-31G* level of theory, the electronic energy of the acetyl cation equals −152.923534 E_h with a ZPE of 0.044775 E_h and the enthalpy of −152.874253 E_h at 298 K. The electronic energy obtained by a geometry reoptimizations at the QCISD/cc-pVDZ level of theory amounts to −152.531426 E_h.

(44) (a) Nobes, R. H.; Bouma, W. J.; Radom, L. *J. Am. Chem. Soc.* **1983**, *105*, 309. (b) Egsgaard, H.; Carlsen, L. *Chem. Phys. Lett.* **1995**, *236*, 78.

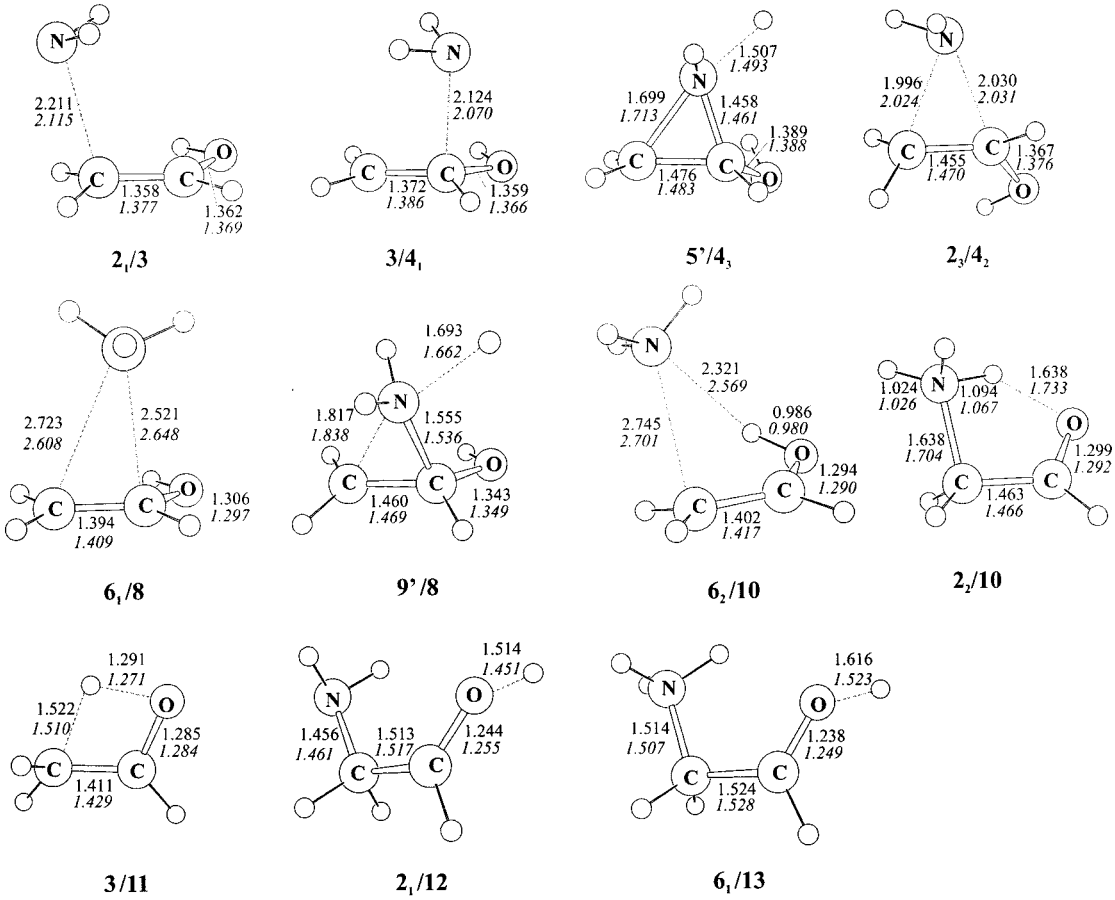


Figure 2. Optimized geometries of the transition structures involved in the rearrangements of **2** and **6**; bond lengths are given in Å (B3LYP results in roman and QCISD in italics).

(187.2 kcal/mol relative to **7**). In contrast, formation of an ammonium ion and **10** makes the latter reaction quite exothermic (−22.8 kcal/mol). However, once more it is not very probable that this pathway will play a role in the enzymatic reaction because the initial step, with an activation enthalpy higher than 29.2 kcal/mol, poses too high of a barrier.

O−H Bond Cleavage of 2 and 6 as the Initial Steps (Scheme 4B and E). From $\mathbf{2_1}$, the homolytic O−H bond cleavage occurs through the transition structure $\mathbf{2_1/12}$. The related energy demand equals 33.9 kcal/mol, and it is unlikely that the enzyme could reduce such a high-energy barrier to make this step feasible. For the sake of completeness, we have, nevertheless, investigated the whole reaction profile. The energy sum of the products, hydrogen and aminoethanal, **12**, lies 20.4 kcal/mol above the reactant structure $\mathbf{2_1}$. If a heterolytic O−H bond cleavage of $\mathbf{2_1}$ were to take place, the energy of the aminoethanal radical anion[45] would be, as expected, even higher (371.0 kcal/mol above $\mathbf{2_1}$). The energy demand for the homolytic C−N bond cleavage in **12** equals 73.2 kcal/mol. If this cleavage is coupled with ammonia formation, the reaction enthalpy drops to −24.7 kcal/mol (relative to **12** + H•). A heterolytic C−N bond cleavage of **12** can be discarded because the energy sum of the products, NH_2 anion and acetyl cation,[43,44] lies 249.3 kcal/mol above **12**. In any case, because the first step of the reaction, the hydrogen abstraction, is energetically quite demanding (33.9 kcal/mol), it is very unlikely that this mechanistic scenario plays a role in the enzymatic catalysis.

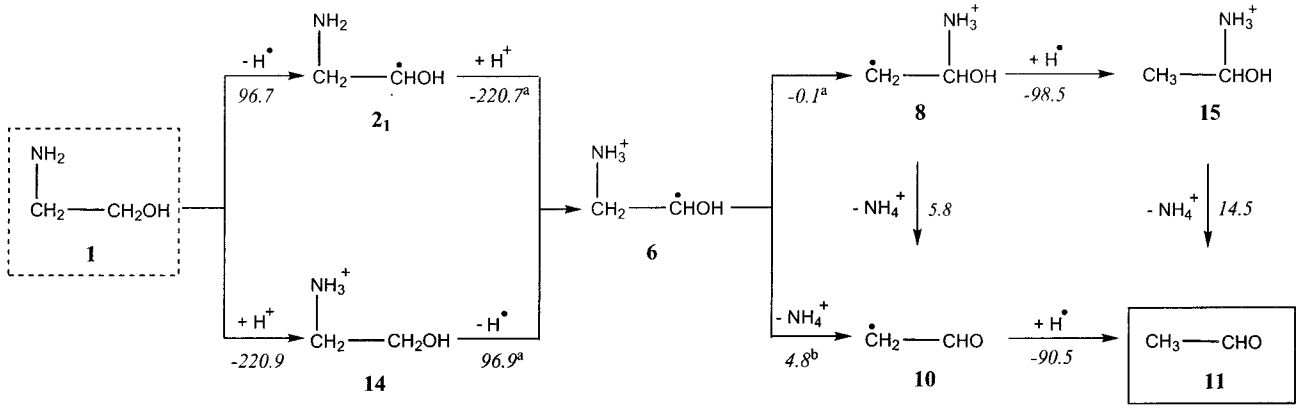
A homolytic O−H bond cleavage[46] in $\mathbf{6_1}$ proceeding through the transition structure $\mathbf{6_1/13}$ would require 38.1 kcal/mol, again too high for an enzymatic reaction. Consequently, the PES involving **13** needs no further detailed discussion. Rather briefly, a heterolytic C−N bond cleavage in **13** yields ammonia and the acetyl cation[43,44] as the products, with a combined energy of 25.3 kcal/mol relative to **13**. A homolytic C−N bond cleavage in **13** is energetically even more demanding, being equal to 98.8 kcal/mol. Even if the latter cleavage is accompanied by the N−H bond formation to generate the ammonium ion in a process that is overall exothermic (−18.6 kcal/mol relative to **13** + H•), that route can also be excluded as the barrier, for the initial step $\mathbf{6_1}$ → **13** cannot be overcome.

Direct Ammonia/Ammonium Eliminations (Scheme 4C and F). In the $\mathbf{2_2}$ conformer, an intramolecular nitrogen−hydrogen interaction renders the direct elimination of NH_3 quite attractive. The transition structure for this path $\mathbf{2_2/10}$ lies 29.6 kcal/mol above $\mathbf{2_2}$; this barrier originates from the cleavage of a strong O−H bond accompanied by the formation of a weaker N−H bond, thus resulting in a relatively high activation enthalpy of the late transition structure $\mathbf{2_2/10}$ in which the N−H bond is

(45) For $NH_2CH_2CHO^{-\bullet}$, the electronic energy at the B3LYP/6-31G* level of theory equals −209.120158 E_h with a ZPE of 0.070674 E_h and the enthalpy of −209.043794 E_h at 298 K. At the QCISD/cc-pVDZ level of theory, the electronic energy amounts to −208.553294 E_h.

(46) As an alternative, the heterolytic O−H bond cleavage of $\mathbf{6_1}$ can be discarded, because the energy of hypervalent aminoethanal radical ($NH_3-CH_2-CH=O^{\bullet}$; the electronic energy on the B3LYP/6-31G* level of theory equals −209.723439 E_h with a ZPE correction of 0.085502 E_h and the enthalpy of −209.632144 E_h at 298 K; at the QCISD/cc-pVDZ level of theory the electronic energy equals −209.166357 E_h) lies 216.3 kcal/mol above $\mathbf{6_1}$.

Scheme 5. Conceivable Pathways for the Conversion of Aminoethanol, **1**, into Ethanal, **11** (Numbers in Italics Correspond to the Reaction Enthalpies in kcal/mol at 298 K of the Corresponding Reactions)



[a] Number corresponds to a reaction in which conformer $\mathbf{6_1}$ is involved. [b] Number corresponds to a reaction in which conformer $\mathbf{6_2}$ is involved.

formed, while O−H is almost completely broken (see Figure 2). While a complex between ammonia and the ethanal radical, **10**, has not been located on the PES, it can be postulated to exist (see next paragraph). However, once more the high activation energy makes the direct loss of ammonia not a very probable rearrangement pathway even if the barrier could become lowered somehow by enzyme catalysis.

The activation enthalpy for the direct elimination of an ammonium ion starting from $\mathbf{6_2}$ via TS $\mathbf{6_2/10}$[47] equals only 10.6 kcal/mol. The transition structure $\mathbf{6_2/10}$ can be discussed in terms of an interaction of NH_3 with the stable enol radical-cation **7**[39] (e.g., compare the bond lengths of $\mathbf{6_2/10}$ and **7**; Figures 1 and 2), where a H-bond between the nitrogen atom and the hydrogen from the OH group exists ($d_{NH} = 2.569$ Å). Further, as already mentioned, a spin delocalization through the C−C−O backbone in $\mathbf{6_2/10}$ stabilizes the transition structure in contrast to $\mathbf{2_2/10}$, where the spin delocalization through three centers is not achievable. A complex between the ethanal radical, **10**, and NH_4^+ has been located with a stabilization energy of 17.6 kcal/mol below $\mathbf{6_2}$. The energy requirement for a complete separation of the two building blocks amounts to 22.4 kcal/mol, indicating a strong electrostatic interaction between NH_4^+ and the carbonyl group. However, in an enzymatic environment, interaction of NH_4^+ with negatively charged amino acid residues may help to pull out NH_4^+ from the active site; in this case, the overall rearrangement costs only 10.6 kcal/mol.

Formation of Ethanal, 11. 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. In an ideal case of complete enzyme saturation, one could approximate k_{cat} with the rate constant of the product formation. In a realistic case, the rate of the product formation would be lower, and thus the activation enthalpy would be higher. Taking the value of $k_{cat} = 55\ s^{-1}$ for the ethanolamine ammonia lyase at 295 K,[48] we derived the activation enthalpy for the rate-determining step from the Eyring equation. Assuming a reasonable range of activation entropy of 0−10 cal/mol K,[17] we found that the activation enthalpy falls into the range 14.9−17.8 kcal/mol, respectively; thus, any process with an activation enthalpy higher than ca. 16 kcal/mol can be discarded as a potential step in the deamination of aminoethanol catalyzed by the enzyme. Therefore, **4** cannot serve as an intermediate in the rearrangement of **1** → **11**, because all of the investigated reactions, in which **4** is involved (Schemes 2 and 3), have activation enthalpies of their rate-determining steps highly exceeding 16 kcal/mol.

Possible steps for the formation of ethanal **11** from intermediates involved in these reactions, which obey the transition barrier limit stated above, are summarized in Scheme 5; energies of the relevant closed-shell species are presented in Table 3S in the Supporting Information. For example, intermediate **10** is accessible via direct loss of NH_4^+ from the protonated radical precursor $\mathbf{6_2}$ (see Scheme 4F); here the corresponding activation enthalpy equals only 10.6 kcal/mol. Considering the energy barrier, we found that this elimination has a high probability of occurring even if full protonation of **2** in the enzymatic process has not yet been demonstrated to take place. Nevertheless, as already shown,[26] also partial protonation can result in a substantial stabilization of the transition structure, thus bringing the energy demand in a region accessible to the enzymatic reaction. From **10**, ethanal, **11**, could be formed by interaction with the 5′-deoxyadenosine, while, at the same time, the active form of the vitamin B_{12} is regenerated.

Intermediate **8** can be formed either by the dissociation/association mechanism (Scheme 2D) or via direct migration of the NH_3 group (Scheme 2F). The former path can be ruled out because of the energy barrier involved in the rearrangement (higher than 16 kcal/mol). In contrast, the latter route $\mathbf{6_1}$ → **8** with an activation enthalpy of only 10.4 kcal/mol has the lowest energy barrier of all investigated rearrangement possibilities. From **8**, ethanal can be formed either by loss of an ammonium ion resulting in **10** or by hydrogen addition from the 5′-deoxyadenosine followed by loss of an ammonium ion.

The proposal for an intramolecular NH_3 migration **6** → **8** (Scheme 2F) is in line with computational findings of quite similar rearrangements catalyzed by diol dehydrase,[16−18,26] while a dissociation pathway related to **6** → **10** (Scheme 4F) is supported by solution experiments.[49] However, the EPR spectra[12] do not allow one to distinguish between intermediates of the rearrangements, thus leaving the question of the actual rearrangement pathway still unanswered. While the calculations slightly favor the NH_3 migration pathway, with an activation-

(47) The optimization of the IRC structure in the direction of product could not converge because of the formation of two separate species. Thus, the energy of the product structure was obtained by combining the energies of the separately optimized structures **10** and NH_4^+.

(48) Faust, L. P.; Babior, B. M. *Arch. Biochem. Biophys.* **1992**, *294*, 50.

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Table 3. Comparison between the Computed and Experimentally Derived Reaction Enthalpies (in kcal/mol)

	B3LYP/6-31G* [a]		QCISD/cc-pVDZ[a,b]		
	$\Delta_r H$ (0 K)	$\Delta_r H$ (298 K)	$\Delta_r H$ (0 K)	$\Delta_r H$ (298 K)	$\Delta_r H_{exp}$
$\mathbf{2_1} \rightarrow \mathbf{10} + NH_3$	−6.8	−5.5	−6.1	−4.8	-4.6 ± 3.2
$\mathbf{6_1} \rightarrow \mathbf{10} + NH_4^+$	6.7	11.9	4.4	5.6	

[a] ZPE correction has been taken into account. [b] Enthalpies obtained by performing the frequency calculations for reactants and products at the QCISD/cc-pVDZ level of theory.

enthalpy difference between the direct NH_3 migration and the NH_4^+ elimination of only 0.2 kcal/mol, a definitive answer cannot be given. From the 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.[50] The estimated energy barrier associated with that reaction step equals 15 kcal/mol at 298 K;[51] thus, both scenarios suggested by the present computational work could well take place in the real enzymatic reaction. All other investigated pathways can be ruled out on energetic grounds.

Reaction Enthalpies − A Comparison of Calculated and Experimental Values. The reaction enthalpies for the transformation of aminoethanol radical, $\mathbf{2_1}$, into ethanal radical, **10**, and ammonia have been calculated at both levels of theory (Table 3). To obtain even more accurate reaction enthalpies, a frequency analysis of the structures in question was performed at the QCISD/cc-pVDZ level of theory as well. As the experimentally derived data for the enthalpy of the aminoethanol radical formation do not seem to exist in the literature, this figure was estimated from other data available.[52] Because the reliability of some of the numbers presented in thermochemical tables[53,54] can be questioned, where available, the enthalpies reported by Cioslowski et al.[55] were used. For protonated aminoethanol **6** and its rearrangement into ethanal radical **10** and ammonium ion, we did not find sufficient experimental data to calculate the corresponding experimental enthalpy; thus, only the computed values are given. The reaction enthalpies calculated at both levels of theory fall into the range of the enthalpy predicted from the experimentally available data. The uncertainty of experimentally derived enthalpies is quite pronounced, and one can safely assume that the computationally determined values, especially at the QCISD level of theory, are even more reliable in this particular case.

Conclusion

The computational study of the aminoethanol rearrangement clearly discriminates between various mechanistic scenarios, thus adding in the elucidation of the actual mechanism of this important enzymatic reaction.

Because of their high activation enthalpies (more than 23 kcal/mol), reaction mechanisms involving complete detachment of the NH_2/NH_3 groups (dissociation/association mechanisms) can be ruled out. Further, isomerization pathways proceeding through cyclic intermediates are unrealistic as well because of the fact that such cyclic structures have not been obtained as minima on the PES neither for the protonated nor for the unprotonated radical. Also, mechanisms involving a stepwise elimination of the NH_3/NH_4^+ species are highly unlikely to play a role in the actual rearrangement routes because of high-energy barriers involved in these pathways.

Concerning the direct transfer of the NH_2 group in the aminoethanol radical, that pathway can be definitively ruled out because of the exceeding high-energy demand (more than 70 kcal/mol). It is inconceivable that any enzyme could reduce such a barrier for this rearrangement to become feasible. On the other hand, the activation enthalpy for a direct transfer of the NH_3 group in the protonated aminoethanol radical is computed to be the lowest (10.4 kcal/mol) of all rearrangement barriers investigated in this study, thus making that pathway the most probable rearrangement mechanism of the enzyme catalysis. Indeed, this finding confirms the earlier hypothesis[15,16,26] that (partial) protonation of the migrating group reduces significantly the energy barrier.

The direct NH_3 elimination in aminoethanol radical is not expected to be the actual rearrangement because of an energetically quite demanding barrier. On the other hand, for the protonated form of the aminoethanol radical, the activation enthalpy for a direct NH_4^+ elimination falls into the range of enzyme catalysis (10.6 kcal/mol). However, the possible complex formation between an NH_4^+ ion and the ethanal radical could present a bottleneck for that particular mechanistic route because of the high-energy demand for the complex dissociation (22.4 kcal/mol). Nevertheless, as mentioned, the enzyme surrounding could help in abstracting the NH_4^+ ion from the active site, thus preventing a complex formation.

Clearly, the X-ray structure of the ethanolamine ammonia lyase would certainly resolve the mechanistic dichotomy in distinguishing between the two mechanistic pathways (i.e., direct NH_3 migration **6** → **8** vs NH_4^+ elimination **6** → **10**) predicted by the present model calculations as the two most probable rearrangements of aminoethanol in enzymatic reactions.

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Supporting Information Available: Tables of electronic energies, zero-point energies, and enthalpies of the stationary points on PES (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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