Journal of Molecular Graphics and Modelling 51 (2014) 173–183

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Stereoelectronic explanations for the mechanistic details of transimination and HF elimination reactions

Hatice Gökcan, F. Aylin Sungur Konuklar*

Istanbul Technical University, Informatics Institute, Computational Science and Engineering Division, Ayazağa Campus, 34496 Maslak, Istanbul, Turkey

ARTICLE INFO

Article history:
Accepted 22 May 2014
Available online 2 June 2014

Keywords:
Density functional calculations
Transimination
HF elimination
Transferases

ABSTRACT

The β-fluoroamines are commonly used as substrate analogs to determine the mechanistic details of enzymatic reactions. Presence of fluorine atom gives rise to the alterations in the electronic profile and the pK_a of molecules which results in mechanistic deviations. The fluorine-substituted mechanism-based substrate analogs are widely used in the inactivation of pyridoxal 5′-phosphate (PLP)-dependent enzymes. The presence of fluorine atom also alters the sequence of reactions taking place in PLP-dependent enzymes where the HF elimination reaction appears in between the transimination and inactivation reactions. Despite the amount of the works on β-fluoroamines, the effect of stereoelectronic differences on the transimination and HF elimination reactions taking place in PLP-dependent enzymes has not been investigated yet. A density functional theory study is conducted to elucidate mechanistic details of the reactions occurring in PLP-dependent enzymes. In order to understand the mechanistic insights of different isomers and the effect of the fluorine atom, 4-amino-3-fluorobutanoic acid (3-F-GABA) enantiomers are chosen to be investigated besides 4-aminobutanoic acid (GABA), which is the natural substrate for γ-aminobutyric acid aminotransferase (GABA-AT). The investigated β-fluoroamines are the experimentally proposed potential inhibitors of PLP-dependent enzyme GABA-AT.

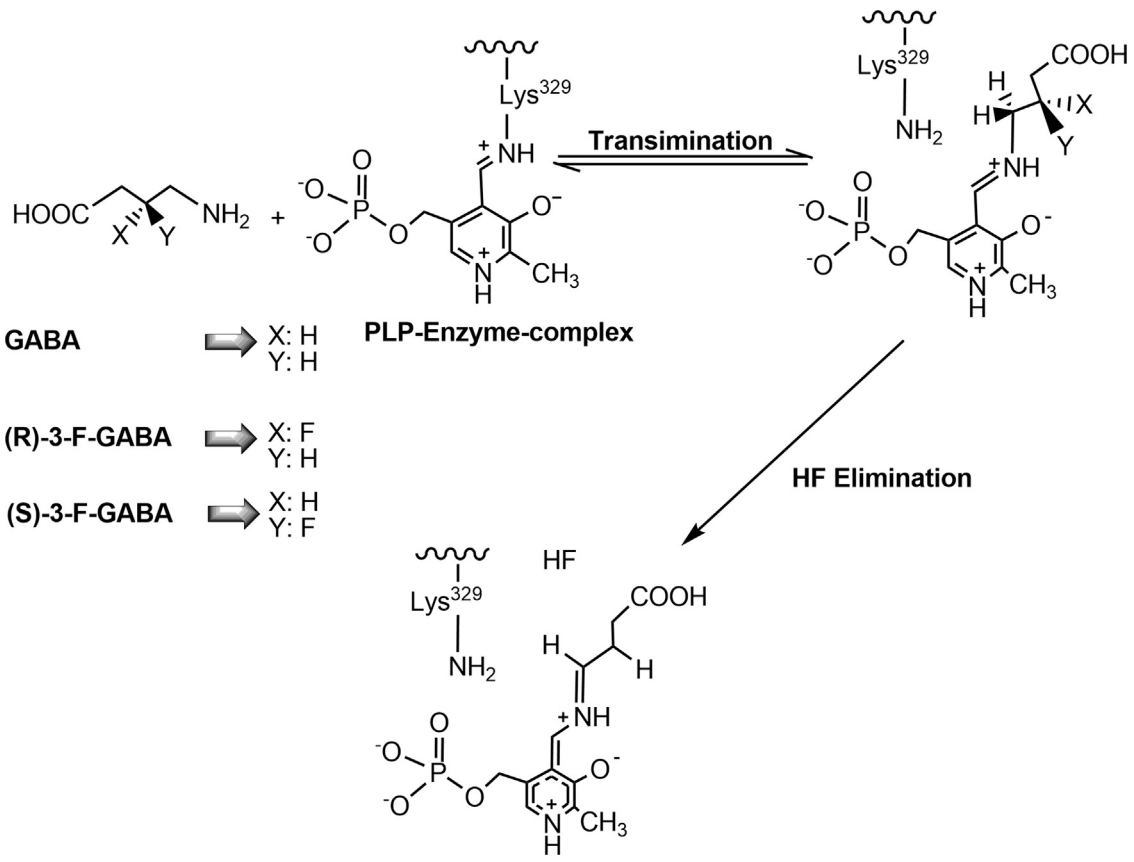
1. Introduction

In medicinal chemistry, β-fluoroamines are widely used as irreversible inhibitors of several enzymes. Additionally, chemists make use of β-fluoroamines as substrate analogs to elucidate the mechanistic insights of the enzymatic reactions. Both the electronic profile and the pK_a of molecules are altered by the presence of fluorine atoms, which results in mechanistic deviations [1]. Moreover, the conformational preferences have critical roles in several enzymatic reactions and the bioactivity of substrates [2–6]. The conformational preferences of β-fluoroimines based on the stereoelectronic arguments were investigated by Sparr et al. [4] in which the conformational preference of (−)gauche over anti is demonstrated to be a consequence of the hyperconjugative interactions between the C—F bond and vicinal C—H bonds ($\sigma_{C-H} \rightarrow \sigma^*_{C-F}$) [4]. In addition, an energy difference between gauche and anti-conformers of the 1,2-difluoroethanes was also associated with the hyperconjugative interactions [7]. Furthermore, the neutral and zwitter-ionic conformations of 3-F-GABA were also studied in solution using both static and dynamic electronic structure calculations [5,8,9].

In PLP-dependent enzymes, the amount of σ–π overlaps in the cofactor-derived Schiff bases determines the specificity of the enzymatic reactions, which corroborates the Dunathans stereoelectronic model [4,10–17]. The cofactor-derived Schiff bases in PLP-dependent enzymes are formed via the transimination reaction (Scheme 1), which involves an internal aldimine where the PLP and ε-amino group of the active site residue is bound covalently [18,19]. During the reaction, the covalent bond between PLP and the enzyme is broken and the new Schiff base, namely external aldimine, is formed with the incoming substrate [19–21]. Moreover, the nitrogen atom on the PLP ring is reported to be protonated to enable further stabilizations via the interactions with nearby residues within the active site [15,22–25]. On the other hand, after the formation of the external aldimine, the chemical reactions have a wide range of diversity based on the specificity of the enzyme and also the nature of the substrate. In the case of fluorine-containing substrates such as β-fluoroamines, the transimination reactions are followed by the elimination of a fluoride ion in the form of HF (Scheme 1) [1,26–29]. The computational studies related to PLP-dependent enzymes are mostly concentrated on the external aldimine formation [18,20,22,30–33]. Additionally, few studies are present on base-induced HF elimination reactions with the systems that involve a pyridine ring [30,34].

* Corresponding author. Fax: +90 212 285 7073.
E-mail address: konuklar@itu.edu.tr (F.A.S. Konuklar).

http://dx.doi.org/10.1016/j.jmgm.2014.05.006
1093-3263/



Scheme 1. Schematic representation of two successive reactions occurring in PLP-dependent enzymes and the model compounds studied through the reaction paths.

In spite of the works on the conformational dynamics of β-fluoroamines [1,5], the effect of the stereoelectronic differences are not investigated for the transimination and HF elimination reactions. In an experimental work, Clift et al. proposed fluorine-substituted GABA enantiomers as potential inhibitors of the PLP-dependent enzyme GABA-AT [1] where GABA (Scheme 1) is known as the natural substrate for GABA-AT. The fluorine-containing analogs (**3–F–GABA**, Scheme 1) could undergo HF elimination after the formation of the external aldimine. The HF elimination reactions may proceed either via the stepwise elimination unimolecular conjugate base (E1cb) or concerted bimolecular elimination (E2) mechanisms [1].

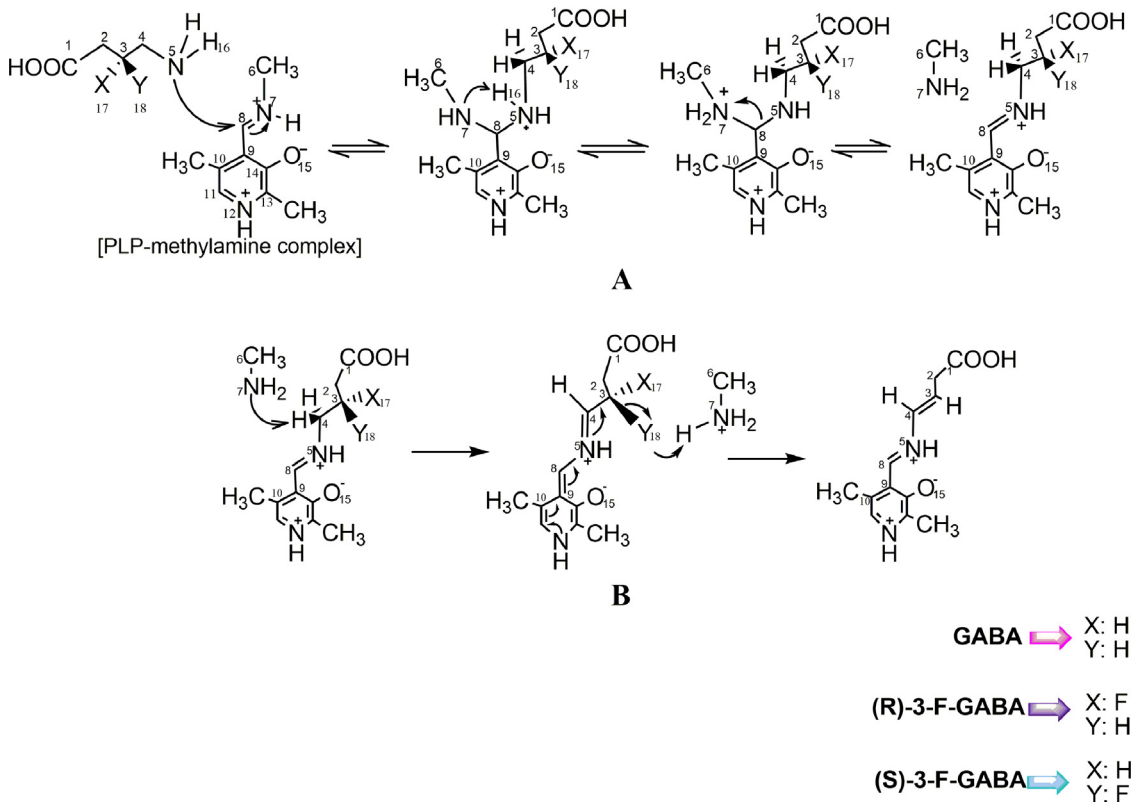
The computational study presented here is a detailed examination of the transimination and HF elimination reactions taking place in the inhibition mechanism of GABA-AT. GABA and 3-F-GABA (R and S enantiomers) are chosen to investigate the mechanistic details of the two successive reactions. The presence of stereoelectronic and steric perturbations enable us to explain the idea behind the inhibition efficiency differences of R and S enantiomers.

2. Computational details

The presence of charged species along the reaction path makes necessary to perform calculations in aqueous medium. Both the geometry optimizations and the frequency calculations in water environment is performed with the B3LYP method and the 6-31 + G(d,p) basis set [35]. The study is conducted using Gaussian'09 (G09) program package [36]. The solvation effect of water is considered by using the polarizable continuum model (PCM) of the Tomasi group as implemented in G09 [37]. The energies that are reported in the text are the thermal free energies obtained with solvent optimization calculations by G09 program package, unless otherwise stated. Moreover, natural bond orbital (NBO) analysis is performed to figure out the importance of stereoelectronic effects on the stability of the structures along the reaction path [38] and the charges reported in the text are based on the NBO calculations. The reactions of GABA and fluorine substituted GABA analogs are studied with a model system where Lys329 residue is represented with a methylamine; which is widely used and accepted representations in literature [9,30,33,39,40]. To reduce the charge delocalization on the pyridine moiety, the nitrogen of the pyridine ring of PLP is modeled as protonated [30,41,42]. On the other hand, the phosphate group of PLP is replaced with a methyl group which is another common representation [30–32,41,43]. The kinetics involved in the R:S binding ratio have been calculated according to the activated complex theory (Boltzman distribution) which describes the relative population at two different states

$$\frac{N_1}{N_2} = \frac{e^{-\Delta G_1/RT}}{e^{-\Delta G_2/RT}}$$

It has been reported that full geometry optimization calculations with higher level of quantum mechanical theories would not change the conformational properties where the energy values may change with the single point energy calculations [44–46]. Therefore, single point MP2 energy calculations with a larger basis set, 6-311 + G(d,p), are performed for the species along the reaction coordinate (see supplementary data) [47]. The graphical representations were obtained using CYLview program [48]. A systematic labeling is applied for the species along the reaction coordinate. Structures that belong to the solution conformers are labeled with lowercase **a** where the bioactive conformers are depicted with lowercase **b**. In addition, the structures that are in their zwitterionic forms are labeled with –**z** and in the presence of water assistance –**w** is used.



Scheme 2. Reaction pathway of (A) Transimination with GABA and fluorine-substituted GABA analogs. (B) HF elimination from fluorine substituted GABA analogs via E1cb mechanism.

3. Results and discussion

In a combined experimental and docking study conducted by Clift et al., the HF elimination and the transimination reactions were investigated based on the binding affinities and relative efficiencies of the GABA and its fluorinated analogs in the presence of PLP-dependent enzyme GABA-AT [1]. It was stated that solution and bioactive conformers of GABA and its fluorinated analogs did not possess the same conformation [1]. Thus, calculations are carried out both for solution and bioactive conformers. Following the conformational analysis of zwitterionic species, the transimination reaction is modeled with the solution and bioactive conformations to discuss the binding efficiencies of the GABA analogs. (Scheme 2A and B). Then the HF elimination is modeled with the products of the transimination rection to investigate the effect of the electrostatic interactions and the stereoelectronic differences on the reaction path.

3.1. Solution and bioactive conformations as zwitterions

In β-fluoroamines, there is a stereoelectronic preference for the C—F and C—N bonds to align *gauche* in order to maximize the stabilizing overlap between the σ^*_{C-F} orbital and the vicinal σ_{C-H} orbital, which is known as stereoelectronic gauche effect [4,7–9,27,49]. In an experimental study on the fluorinated substrate analogs of GABA-AT, a gauche alignment was proposed for the C—F and C—NH_3^+ bonds for the binding conformation of (R)-3-F-GABA where an anti-alignment was suggested in the binding conformation of (S)-3-F-GABA [1]. The relative free energies of the conformers of GABA, (R)-3-F-GABA and (S)-3-F-GABA and the Newman projections of the corresponding conformations are given in Fig. 1. It is found that the stability of both gauche and anti-conformers of GABA (**00Ga-z**, **00Gb-z** and **00Gc-z**, Fig. 1) are almost the same,[1] which is consistent with previous works on GABA [10] and ethylimines [4]. Among the gauche conformations of (R)-3-F-GABA, solution conformer (**00Ra-z)** is lower in energy relative to the bioactive conformer (**00Rb-z**). The energy difference (1.4 kcal/mol) may stem from the stronger electrostatic interaction between the imine hydrogen and the fluorine lone pairs in **00Ra-z,** where similar results were reported before for β-fluoroamines and β-fluoroalcohols [4,50–52]. The anti-conformer (**00Rc-z**) is found to be the least stable conformer. In case of S enantiomer, both the gauche conformers (**00Sa-z and 00Sc-z**) are found to be more stable than the anti-conformer (**00Sb-z**), which is known as the bioactive conformer. An energy difference on behalf of gauche preference is absolutely observed in all cases which is consistent with the stereoelectronic gauche effect [4,7–10,27,49]. However the bioactive conformers are not located as the global minimum structures for the fluorine substituted GABA analogs. Based on the relative free energies, the relative population of **00Rb-z** will be higher than that of **00Sb-z,** which may promote the inhibition efficiency of R.

Experimental studies have also pointed out that high concentrations of substituted GABA analogs were required to inhibit the GABA transamination [1]. The energy differences found between conformations of the 3-F-GABA relative to the equally populated solution and bioactive conformers of GABA might be the possible reason for the required high concentrations during the inhibition. It is stated that substrates that are in zwitterionic form should undergo a deprotonation of the ammonium group before inactivation mechanisms take place [1]. The alterations in pK_a values of substrate species may also influence the reactivity efficiencies as

[1] Small energy differences between enantiomeric pairs of structures were not considered significant, and merely reflect slight differences in the torsion adopted by the CH_2COOH group during optimization; at higher levels of theory they were not observed (see supplementary data).

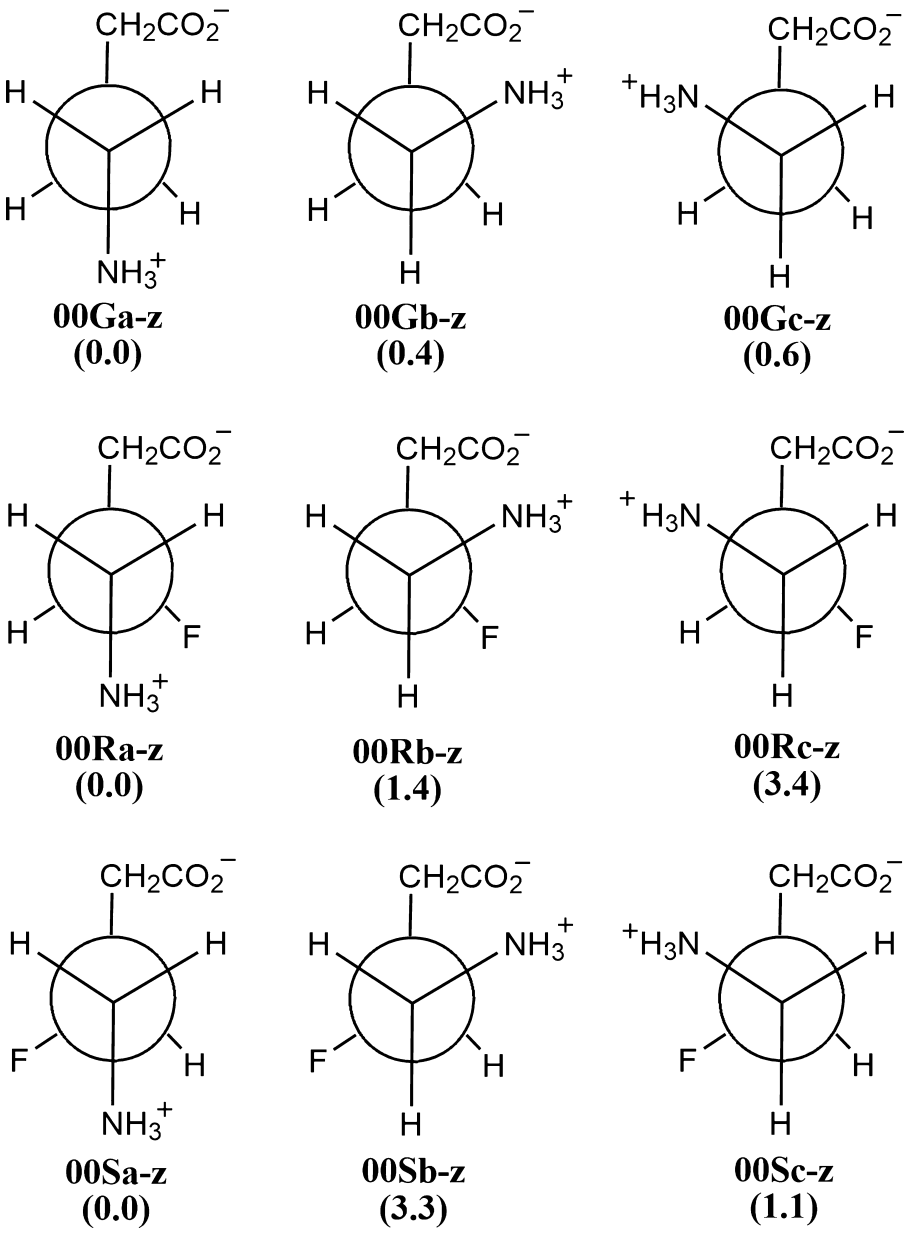


Fig. 1. Newman projections of GABA conformations (**00Ga-z**, **00Gb-z** and **00Gc-z**), (R)-3-F-GABA (**00Ra-z**, **00Rb-z** and **00Rc-z**) and (S)-3-F-GABA (**00Sa-z**, **00Sb-z** and **00Sc-z**) in the zwitterionic form. Relative free energies (kcal/mol) are given in parentheses.

stated by O'Hagan et al. [8]. However, pK_a calculations require a more sophisticated study which is not in the scope of this paper.

3.2. Transimination reactions

Transimination reaction proceeds through the following steps: nucleophilic attack of substrate amine to form geminal diamine; 1,3 proton transfer between the amino groups of substrate and methylamine; and external aldimine formation via the cleavage of the enzyme-PLP covalent bond. Energy profiles of transimination reactions of bioactive conformations of GABA, (R)-3-F-GABA and (S)-3-F-GABA are depicted in Fig. 2 and are calibrated relative to the solution conformer of GABA (**00Ga**), which is the lowest-energy structure among the whole conformers. The energy profile of the binding step of solution conformers is also displayed in the box in Fig. 2. It has been suggested that the substrates in the zwitterionic form undergoes a deprotonation at the beginning of the transimination reaction where Thr353 residue is proposed for the assistance of the process due to its proximity to the phosphate group of PLP in the active site [1]. The deprotonation before the binding lowers energy differences among the conformers relative to the corresponding zwitterionic forms (Fig. 2). The nucleophilic attack of the amine group of substrate to C8 of the PLP-methylamine complex is known as the binding step, which regulates the enzymatic activity [1]. It is important to analyze the binding step energy barrier in order to understand the dynamics of the substrate-binding efficiencies. The binding of the bioactive conformer of GABA (**00Gb**) to the GABA-AT is modeled with the transition state **00TS01Gb** (Fig. 3), having an energy barrier of 26.2 kcal/mol, which is higher than the solution conformer **00TS01Ga** by 2.1 kcal/mol (Fig. 2). The higher barrier value of **00TS01Gb** can be attributed to the different nature of the steric crowdance around the reacting center. The transition state yields **01Gb,** which has a relative free energy of 23.8 kcal/mol (Fig. 2). In our previous work on the fluorinated aromatic substrate, the binding step barrier is calculated to be 14.0 kcal/mol with N7-C8 distance being 2.133 Å [30]. Although

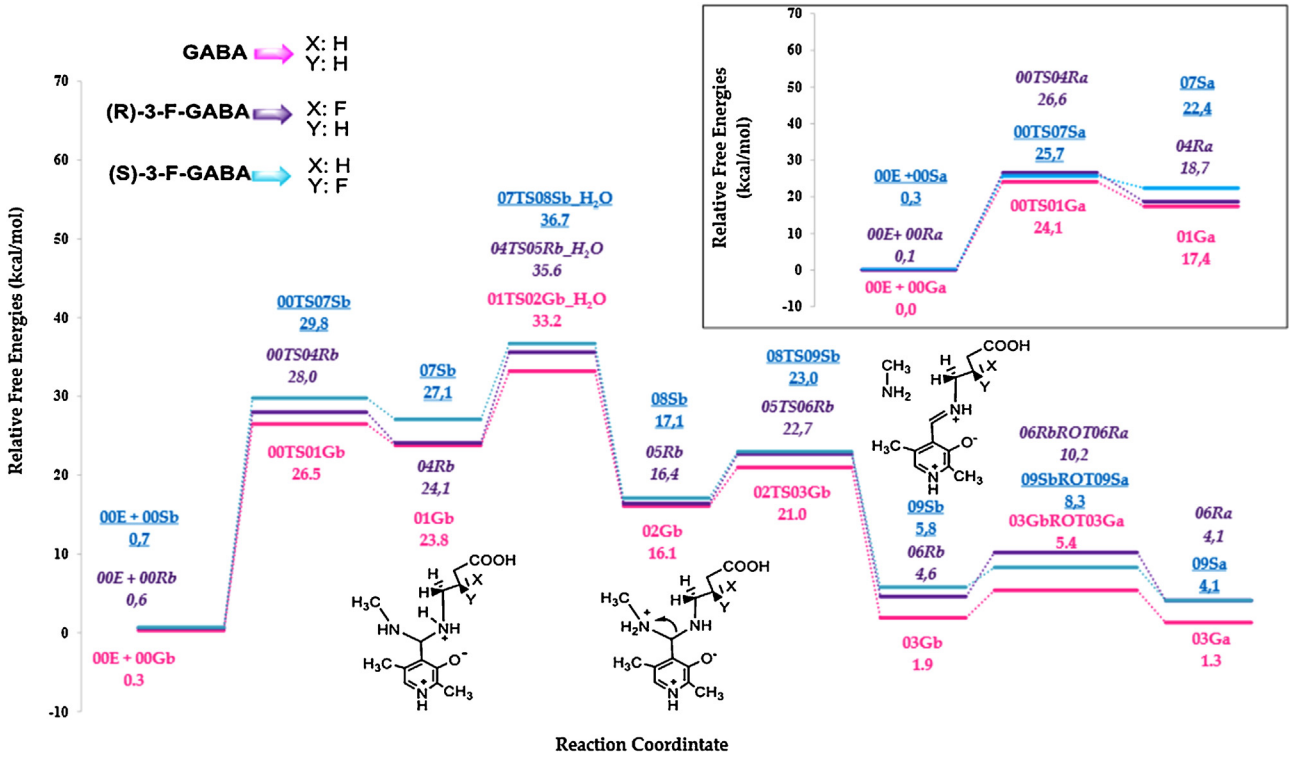


Fig. 2. The energy profiles of transimination reactions of GABA (pink), (R)-3-F-GABA (purple) and (S)-3-F-GABA (blue).

the steric hindrance created by the aromatic substituent is greater, the conformational preference of the carboxylic group in the previous transition state structure results in stronger hydrogen bonding interactions, thereby lowering the barrier height.

In the case of 3-F-GABA analogs, the binding step barriers for the bioactive conformers (**00Rb** and **00Sb**) are calculated to be 27.4 kcal/mol, and 29.1 kcal/mol yielding **04Rb** and **07Sb** respectively (Fig. 2). In both cases, the solution analogs have lower energy barriers. However, the order of energy barriers of the binding step reversed going from solution to bioactive conformers. The presence of a long range interaction between the hydrogen atom on the N5 and the fluorine atom (2.435 Å) lowers the energy of the **00TS04Rb** relative to **00TS07Sb** as well as the charge-dipole interactions [53,54]. In addition, the energetic stability of R enantiomer over S enantiomer can also be explained by the stabilizing electrostatic interactions between the C—F bond and electropositive centers ($F^{\delta-}$-C_{β}-C_{α}-$N^{\delta+}$) [55,56].

Experiments indicated that the inhibition efficiency of the R-isomer is at least 10 times greater than the S-isomer [1]. Hence, the binding ratios are calculated for both of the solution and bioactive conformers of 3-F-GABA and compared with the experimental results. The binding ability of the S enantiomer of the solution conformer is found to be higher than that of the R at different levels of calculations which is inconsistent with the experimental results [1]. However the calculated binding efficiencies of bioactive conformers ((R)-3-F-GABA:(S)-3-F-GABA = 19:1) based on B3LYP/6-31 + G(d,p) level reflect the experimental (R)-3-F-GABA:(S)-3-F-GABA binding efficiency ratio which has been proposed to be 10. The ratio obtained from MP2 single point energy calculations (9:1) also supports the binding efficiency of R over S. Thus, succeeding calculations are conducted with the bioactive conformers.

The free energy barrier of the 1,3 proton transfer step for the bioactive conformer of GABA (**01TS02Gb_w,** Fig. 3) is calculated as 9.5 kcal/mol (Fig. 2). In the previous work, the barrier height of the 1,3 proton transfer step without water assistance was calculated as 21.8 kcal/mol, whereas it was found to be 17.2 kcal/mol with the water-assisted mechanism [30]. Herein, the energy barrier obtained for the GABA is lower than both barriers of the previous study [30]. The difference may stem from the conformational preference of the aromatic substrate, which creates electrostatic destabilization based on the nearby oxygen atoms around the reacting center. The fluorinated analogs, **04TS05Rb_w** and **07TS08Sb_w** (Fig. 3), have free energy barriers of 11.5 kcal/mol and 9.6 kcal/mol, respectively (Fig. 2), which are also lower than the previous results [30]. The NBO calculations pointed out that the electrostatic interaction between the lone pairs of C9 and C10 is stronger for the **04TS05Rb_w** than both for the **01TS02Gb_w** and **07TS08Sb_w** structures. Eventhough **04TS05Rb_w** has the highest electrostatic interaction, the transition state structure is destabilized by the presence of the fluorine atom next to the oxygen of the water molecule (3.420 Å) which results in higher free energy value than **01TS02Gb_w**. The transition states of fluorine- substituted analogs yield the structures **05Rb** and **08Sb** with relative free energies of 16.4 and 17.1 kcal/mol, respectively. The charges on the reacting-site atoms have similar values in all transition state structures. Moreover, the MP2 single point energy calculations lowered the energy barrier values of the 1,3 proton transfer step about 3 kcal/mol, yet did not change the rate-determining step. The results indicate that the stereoelectronic differences affect the activation energy barrier.

The last step of the transimination reaction is the removal of the methylamine to form the substrate–PLP complex. The structure **02TS03Gb** (Fig. 4) has a free energy barrier of 4.9 kcal/mol (Fig. 2). The transition states for R (**05TS06Rb**, Fig. 4) and S (**08TS09Sb**, Fig. 4) enantiomers have almost the same free energy barriers (6.3 kcal/mol and 5.9 kcal/mol respectively), which is compatible with previous works [30,41]. The stability of **02TS03Gb** is simply due to the electrostatic interactions of PLP ring carbon (C9) and the reaction-center carbon atom (C8). These electrostatic interactions are weaker in **05TS06Rb** and **08TS09Sb**. After the formation of structures **03Gb**, **06Rb** and **09Sb**, rotations of 120° around the

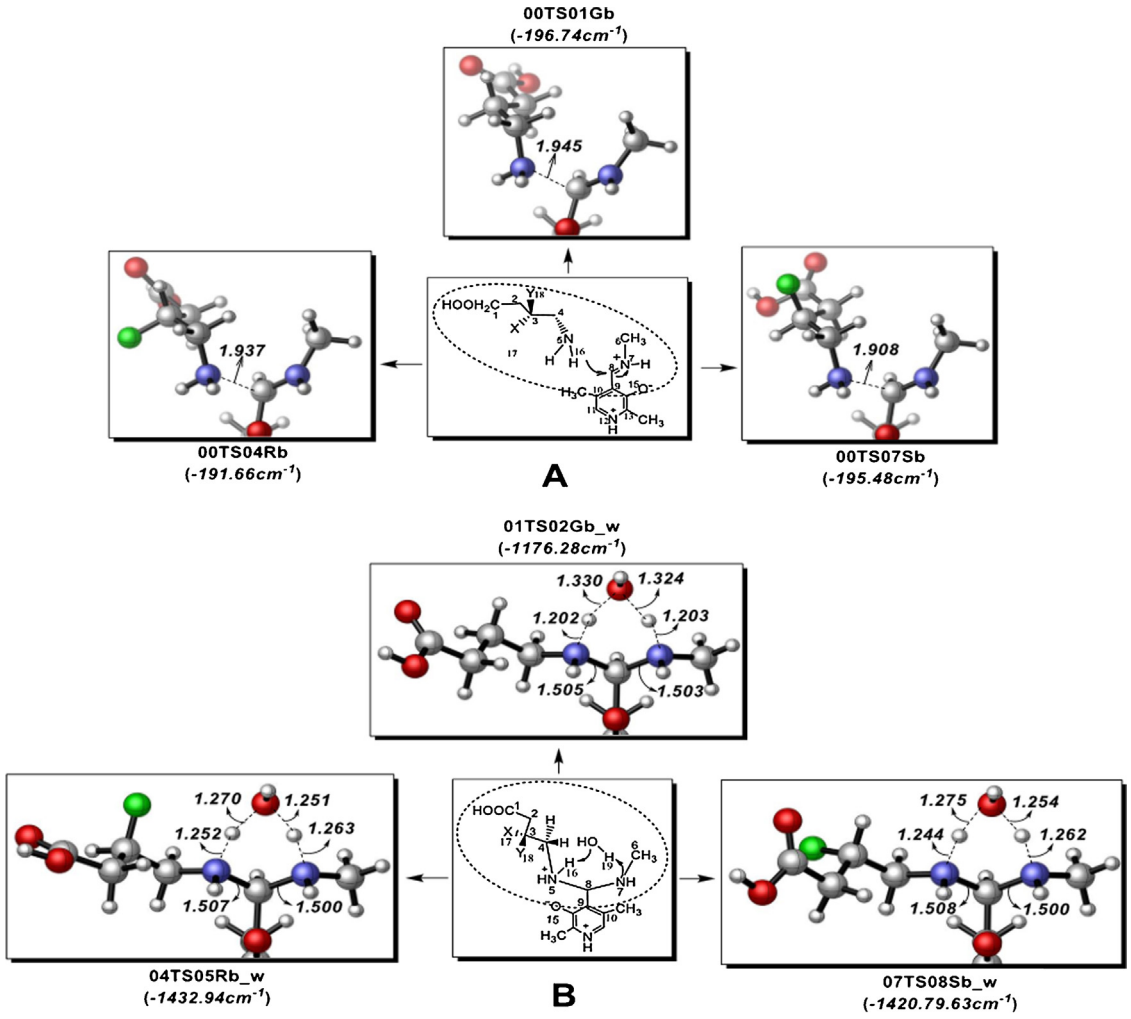


Fig. 3. Three-dimensional geometries of the first two steps of the transimination with the solution conformers of GABA (**00TS01Ga** and **01TS02Ga**, respectively), (R)-3-F-GABA (**00TS04Ra** and **04TS05Ra**, respectively) and (S)-3-F-GABA (**00TS07Sa** and **08TS09Sa**, respectively). (A) Formation of substrate-PLP-methylamine complex. (B) 1,3 proton transfer between nitrogens of substrate and methylamine. The imaginary frequencies that define transition states are given in parentheses.

C3–C4 bond are required for further enzymatic reactions [1]. The free energy barrier for rotations are around 5 kcal/mol, yielding the structures **03Ga**, **06Ra** and **09Sa**. The MP2 single point energy results drastically change the rotational energy barrier values to the point that the barriers are almost doubled or tripled. However, the mechanistic conclusion was not affected by the variations in the barrier heights.

3.2.1. Fluoride ion elimination

The fluorine-substituted substrate analogs are still under investigation, not only due to the conformational dynamics but also due to their prominent efficiency on the enzymatic reactions. Even though the experimental [1,4,20,57] and theoretical [30,34,58] studies have provided some clues regarding the fluorine-containing substrate analogs, the effect of the configurational changes of the substrates on the enzymatic reactions is still not explained. The alignment of C—F and C—N bonds would lead to different consequences for the HF elimination reaction, especially if the mechanism is restricted by stereoelectronic circumstances [1,34]. The effect of configurational and stereoelectronic differences on the HF elimination reaction can be explained computationally via model reactions of (R)-3-F-GABA and (S)-3-F-GABA.

It is known that the HF elimination mechanisms may proceed via either a stepwise E1cb or a concerted E2 mechanism. These elimination reactions are typical examples of the base-induced β-elimination reactions with the formation of a C—C double bond [44]. In both theoretical and experimental studies, it is proposed that the stepwise E1cb mechanism is generally assisted by a residue located near the active site, particularly a nearby Lys residue [1,30,59]. In a similar way, the concerted E2 elimination in enzymes, would likely be assisted either by water or by water and a nearby basic residue together [26,30,58]. Lys329 is reported as a candidate base for assistance of the E2 elimination reaction in GABA-AT [1,59]. It is also noticeable that the protonated nitrogen on the pyridine ring of PLP may provide extra stabilization during the elimination reactions [30,42,60].

The first step in the E1cb mechanism is the abstraction of the γ proton at C4. The abstraction of the proton is then followed by HF elimination via proton transfer to the fluorine atom from the $CH_3NH_3^+$ moiety. The energy profile of HF elimination from the (R)-3-F-GABA-PLP complex is given in Fig. 5. The transition state structure (**06TS10R,** Fig. 6) of the proton abstraction step in the E1cb mechanism is modeled with a free energy of 30.7 kcal/mol (Fig. 5). The distance between the transferring proton and C4 is calculated as 1.517 Å, while the distance between the transferring proton and N7 is 1.232 Å. The free energy barrier is calculated as 26.6 kcal/mol, while it was calculated as 7.8 kcal/mol in the previous work with a different substrate analog which is consist of a six-membered ring [30]. This energy difference might stem from the higher charge delocalization ability of the aromatic ring

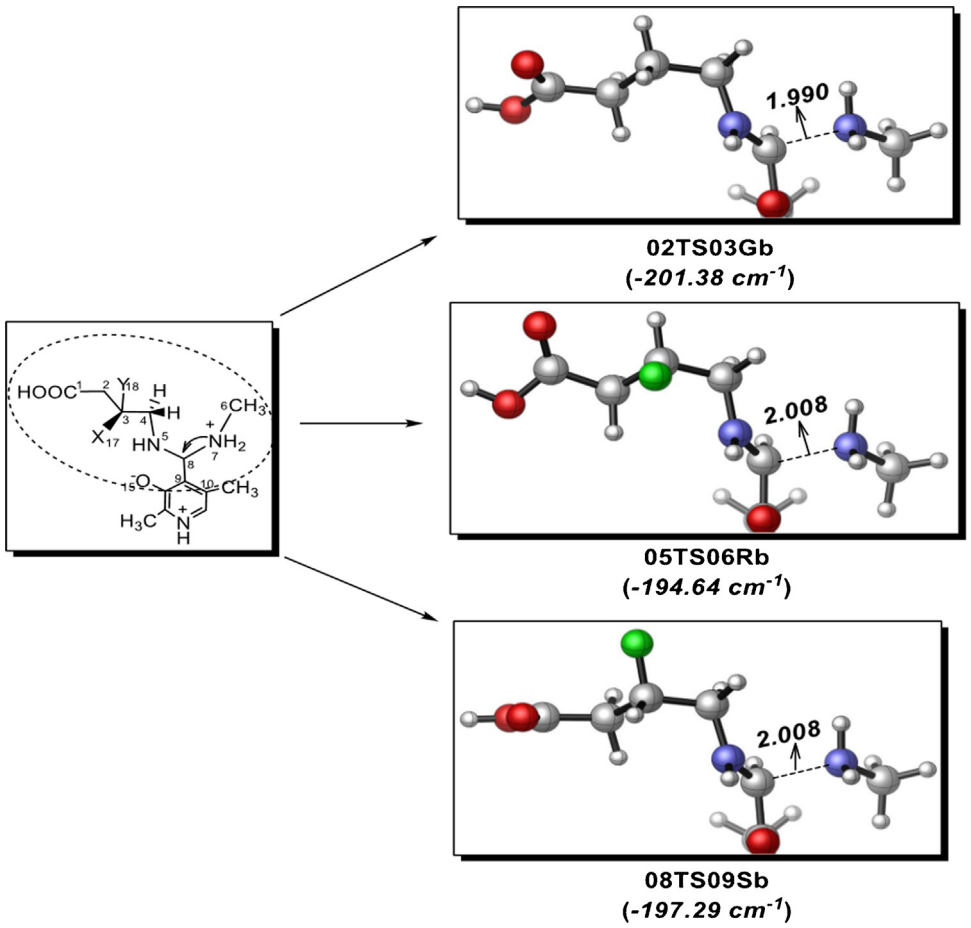


Fig. 4. Three-dimensional geometries of transition state structures of the removal of methylamine with GABA (**02TS03Gb**), (R)-3-F-GABA (**05TS06Rb**) and (S)-3-F-GABA (**08TS09Sb**). The imaginary frequencies that define transition state are given in parentheses.

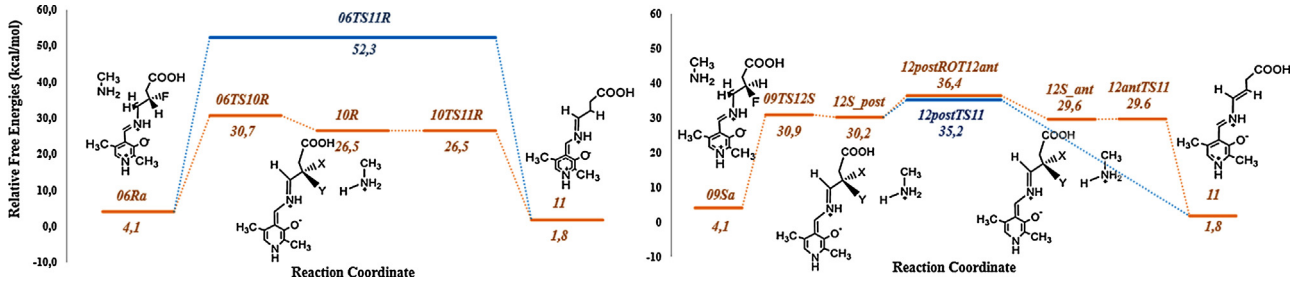


Fig. 5. The energy profile of HF elimination from the (R)-3-F-GABA-PLP complex (left) via E1cb (orange) and E2 (green) mechanisms and HF elimination from the (S)-3-F-GABA-PLP complex via E1cb mechanism (right) with the first scenario (blue) and the second scenario (purple). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

bearing substrate, which would facilitate the proton abstraction in the previous work [30]. The proton abstraction from structure **06Ra** yields the intermediate **10R** (Fig. 6), having a relative free energy of 26.5 kcal/mol (Fig. 5) with a clockwise rotation 93.9° around the C4-N5 bond. The substrate tends to be planar to the molecular plane of PLP ($\beta_{C8\text{-}N5\text{-}C4\text{-}C3}$ = 10.1°) in structure **10R**, while it prefers to be perpendicular in **06Ra** ($\beta_{C8\text{-}N5\text{-}C4\text{-}C3}$ = 104.0°). It should be noted that the F atom is found to be anterior to the molecular plane of PLP in **10R**, while it is posterior in **06a**.

The proton transfer from $CH_3NH_3^+$ moiety to the fluorine atom is rather problematic, and a possible transition state structure could not be modelled [30]. Presence of a shallow minimum due to the looseness of the C—F bond prevents modeling the transition state structure in a solution environment [30,34,58]. To our best knowledge, structure **10TS11R** (Fig. 6) is the first transition state structure that could be located for the HF elimination following E1cb mechanism. According to the solvent optimization calculations, the relative free energy of **10TS11R** is calculated as 26.5 kcal/mol (Fig. 5). Since structure **10R** corresponds to a shallow minimum (Fig. 5) owing to the looseness of the C—F bond (1.485 Å, Fig. 6), the transition state occurs barrierless. Product **11** has a relative free energy of 1.8 kcal/mol (Fig. 5) and the dihedral angle $\beta_{C8\text{-}N5\text{-}C4\text{-}C3}$ is calculated as 25.8°. The overall path of the (R)-3-F-GABA-PLP complex is found to be endothermic.

Clift et al. proposed that, the (R)-3-F-GABA structure does not conform an anti-periplanar arrangement of the eliminating substituents it is not possible to achieve an E2 mechanism. Therefore an alternative concerted mechanism is proposed for the HF elimination from (R)-3-F-GABA-PLP complex with the water assistance. During the course of the reaction an explicit water molecule is used

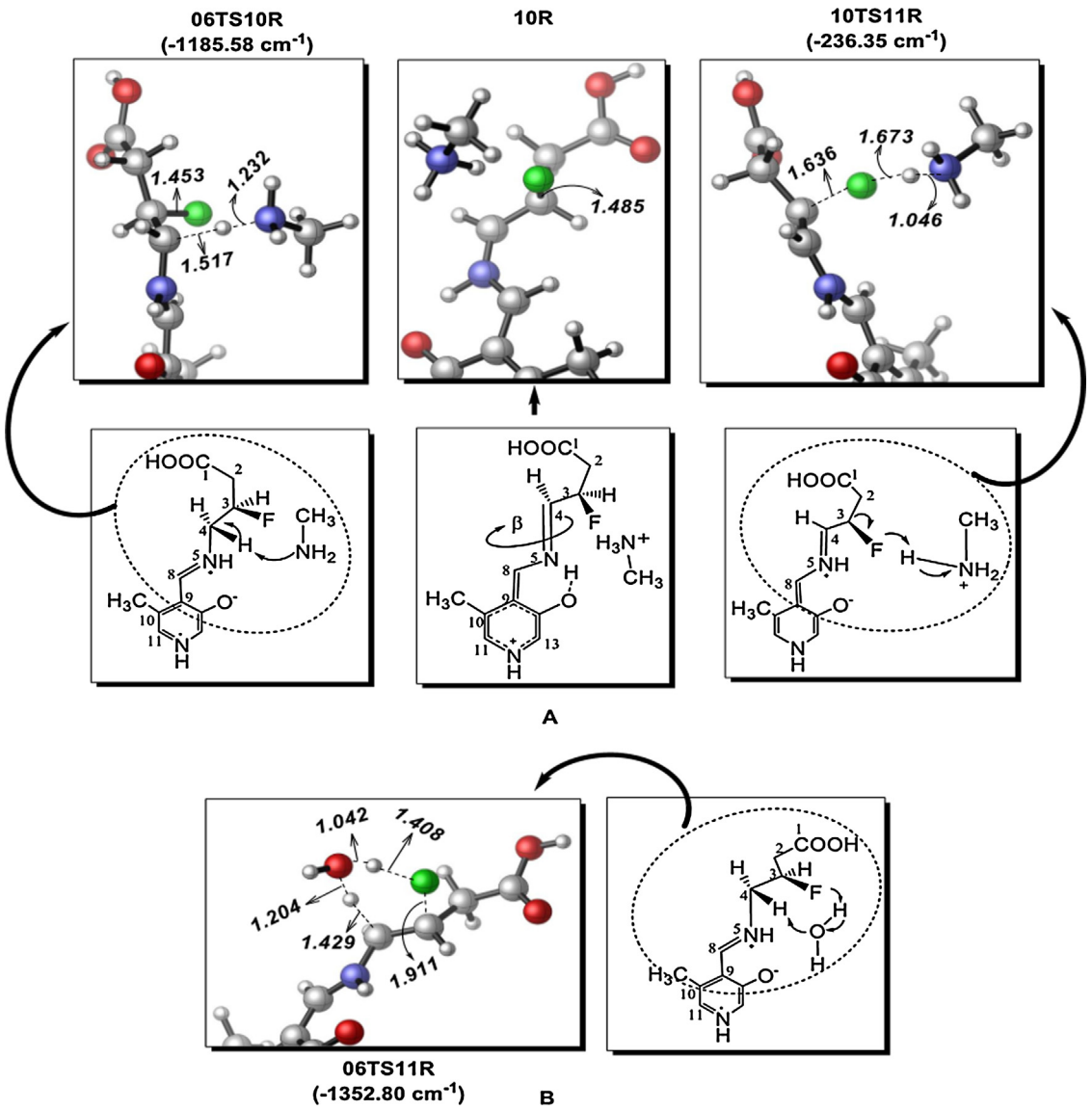


Fig. 6. Three-dimensional geometries of HF elimination reactions of (R)-3-F-GABA-PLP complex. (A) HF elimination via stepwise E1cb mechanism with the assistance of methylamine. (B) HF elimination via concerted E2 mechanism with the assistance of one water molecule. The imaginary frequencies that define transition states are given in parentheses.

as a proton shuttle for the γ proton abstraction. The free energy barrier for the formation of the **11** via the transition state **06TS11R** (Fig. 6) is calculated as 48.2 kcal/mol in the concerted mechanism (Fig. 5) The energetics of reaction paths reveals that there is a preference of the E1cb mechanism over the concerted mechanism in case of (R)-3-F-GABA-PLP complex.

In case of (S)-3-F-GABA, the E1cb mechanism is modeled in a similar way as in (R)-3-F-GABA. The energy profile of HF elimination reactions of (S)-3-F-GABA is given in Fig. 5. The abstraction of the γ proton step, which is achieved with transition structure **09TS12S** (Fig. 7), has a relative free energy of 30.9 kcal/mol similar to the R conformer (**06TS10R,** Fig. 6). Abstraction of the proton from **09Sa** yields the structure **12post** (Fig. 7), in which the F atom is posterior to the molecular plane of PLP. It is observed that in the structure **12post,** the carboxyl group of substrate is almost planar to the molecular plane of PLP and positioned in the opposite direction to the O atom of PLP as in **10R** (Fig. 6).

Two different scenarios can be proposed for the fluoride ion elimination since the fluorine atom and the protonated methylamine are in the opposite direction of the molecular plane of PLP. In the first scenario, the fluoride ion elimination occurs with the assistance of water molecules as proton shuttles. In the second scenario, in order to eliminate the fluoride ion in the form of HF, a rotation around the C3–C4 bond is required. The rotation allows the fluorine atom to be anterior to the molecular plane of PLP.

The transition state structure **12postTS11** (Fig. 7) having a relative free energy of 35.2 kcal/mol is modeled for the first scenario (Fig. 5). The distance between C3 and F is calculated as 1.704 Å, while the distance between the transferring proton and the O10 of water molecule is found to be 1.637 Å. The dihedral angle $\alpha_{N5\text{-}C4\text{-}C3\text{-}C2}$ in structure **12postTS11** shifted by ∼10° relative to **12post** and then rotate back to its original position in structure **11** to maintain planarity of the carboxyl group relative to the PLP ring.

As for the second scenario, the proposed rotation around the C3–C4 bond is achieved with the transition state **12postROT12ant** having a relative free energy of 36.4 kcal/mol (Fig. 5) and yielding the intermediate structure **12ant** (Fig. 7). The dihedral angle $\alpha_{N5\text{-}C4\text{-}C3\text{-}C2}$ is calculated as 61.9° for **12ant** which was −178.0° in the case of **12post**. The proposed scenarios have almost equal

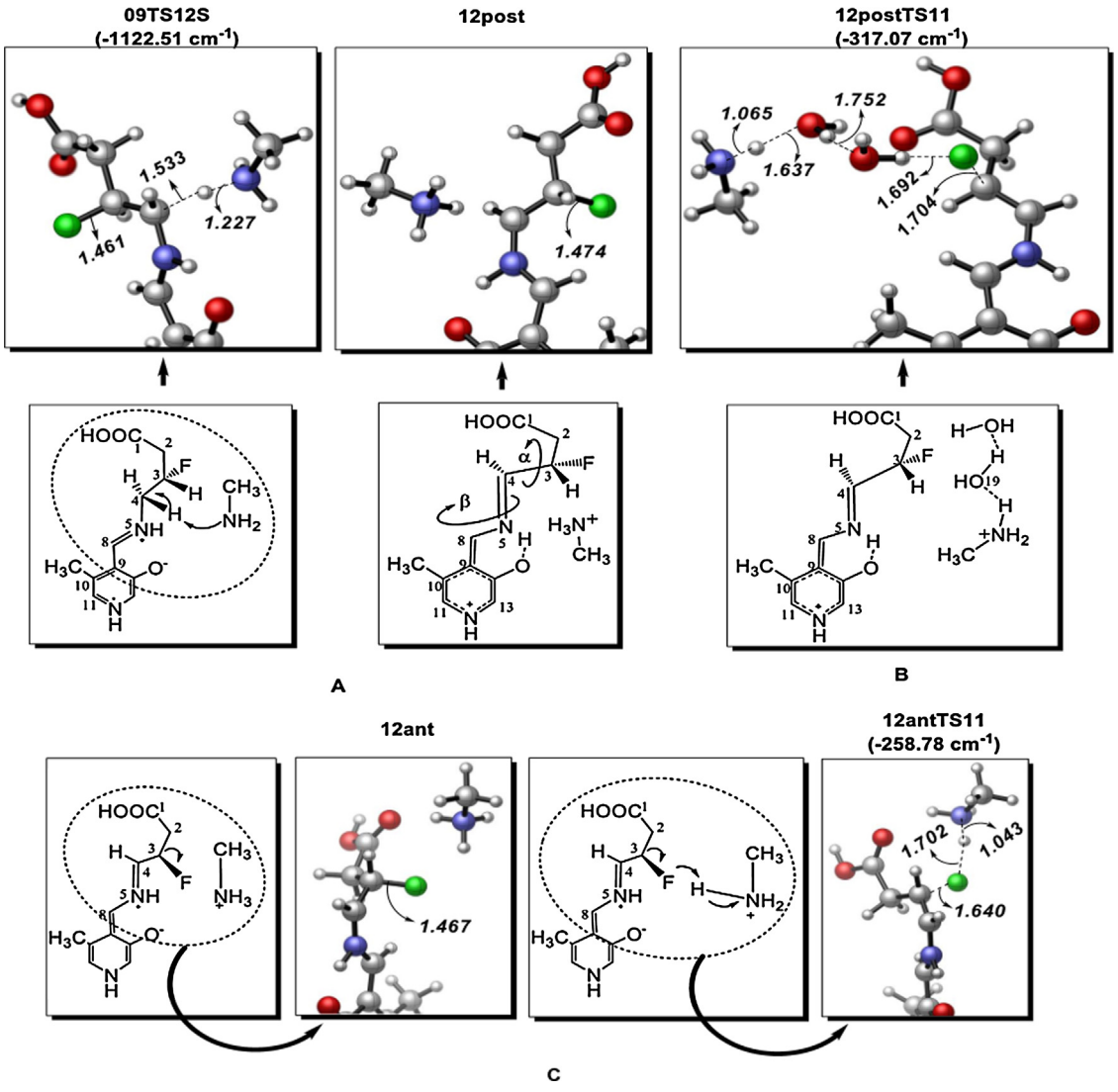


Fig. 7. Three-dimensional geometries of stationary structures of fluoride ion elimination reactions via E1cb mechanism of (S)-3-F-GABA-PLP-complex. (A) Abstraction of γ proton and formation of structure 12post. (B) First scenario of fluoride ion elimination with the assistance of methylamine and water molecules. (C) Second scenario of fluoride ion elimination in the form of HF with the assistance of methylamine. The imaginary frequencies that define transition states are given in parentheses.

probabilities to occur with a relative free energy difference of 1.2 kcal/mol. The results are also supported by the MP2 single point energy calculations. The stronger C3–F bond in structure **12ant** (1.466 Å) relative to the **10R** (1.548 Å) is expected to lower the relative energy. However, the strength of long range interactions predominates in structure **10R** thereby generating 3.0 kcal/mol energy difference relative to structure **12ant**. The last transition state structure **12antTS11** (Fig. 7) of the second scenario is found to be similar to that of R analog (**10TS11R,** Fig. 6). The relative free energy of the transition state structure **12antTS11** is found to be 29.6 kcal/mol (Fig. 5) where the transition state occurs barrierless as in **10TS11R**. A relative free energy difference of 3.1 kcal/mol is calculated between the transition state structures of R and S analogs.

4. Conclusion

The mechanistic details of two successive reactions, transimination and HF elimination, are studied computationally with different conformers of GABA and its fluorine-substituted analogs. The stereoelectronic differences between the structures enabled us to discuss the possible dependencies of the studied reactions.

The dynamics of the enzymatic reactions depend not only upon the activation energy barriers of the transition state structures but also on the conformational and stereoelectronic preferences of the corresponding reactions, as well as the binding efficiencies of the substrates. Prior to the reaction mechanisms, the stability of the solution and bioactive conformers is investigated and it is found that the bioactive conformers of the two substituted GABA analogs are not the global minimum structures. Hence, the relative population of the bioactive conformer of the (S)-enantiomer will be less than that of the (R)-enantiomer. The results indicate that the poor efficiency of the (S)-enantiomer may stem from the instability of the corresponding bioactive conformer. The bioactive conformers of the fluorine substituted analogs are not the global minimum structures to this respect the requirement of the high concentrations of fluorine substituted analogs to inhibit GABA-AT can be explained.

The binding efficiencies of the different enantiomers was also investigated for further explanations. The dynamics of the binding step, which is the first step in the transimination reaction, is analyzed to explain the binding efficiencies. It is found that the R conformer is more convenient for binding. The binding ratio calculated from the relative energy barriers of the bioactive

conformers of (R)-3-F-GABA: (S)-3-F-GABA is found to be 19.1:1 which is in harmony with the experimental work. Results point out the prominence of reactive conformers rather than the most populated substrate analog in the inhibition reactions involving PLP.

In transimination reactions, the stereochemical differences of the fluorine-substituted analogs affect the activation energy barriers by means of electrostatic interactions. Herein, the rate-determining step is found to be the 1,3 proton transfer in the transimination reaction. The differences between the activation energy barriers of the fluorine substituted GABA analogs are not very distinctive. Therefore, the poor performance of the (S) enantiomer cannot be correlated to the energy profile of the transimination. The HF elimination reactions of fluorine-substituted analogs are also important to elucidate the binding preference of GABA-AT and to analyze the reason for the poor performance of (S)-3-F-GABA, which is presented experimentally [1]. The first step of the E1cb mechanism of the (R)-enantiomer (**06TS10R**) and (S)-enantiomer (**09TS12S**) have same activation energy barriers, where the carbanion intermediate of the (R)-enantiomer (**10R**) is more stable. For R enantiomer, a barrierless transition state **(10TS11R)** is obtained for the last step of the HF elimination. Two different scenarios are taken into account for the HF elimination in the (S)-isomer case. It is found that the rotational transition state (**12postROT12ant**) and the transition state involves water molecules as proton shuttles (**12postTS11**) have similar relative free energies being higher than the first step of elimination. The last step of the path is the distinctive for the HF elimination efficiency differences for the enantiomers. The fluoride ion release from the (R)-enantiomer is faster than the (S)-enantiomer supporting the lower HF elimination rate of the (S)-enantiomer, yet more realistic results can be obtained by involving the surrounding amino acids in the active site.

Acknowledgements

We thank ITU-BAP (Grant No: 37011) for generous financial support. Computing resources were provided by the National Center for High Performance Computing of Turkey UHEM (Grant No: 20302008).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jmgm.2014.05.006.

References

[1] M.D. Clift, H.H. Ji, P. Deniau, D. O'Hagan, R.B. Silverman, Enantiomers of 4-amino-3-fluorobutanoic acid as substrates for gamma-aminobutyric acid aminotransferase. Conformational probes for GABA binding, Biochemistry 46 (2007) 13819–13828.
[2] M.B. Smith, Organic Synthesis, McGraw-Hill, New York, 1994.
[3] J.B. Kay, J.B. Robinson, B. Cox, D. Polkonja, 1,2-Disubstituted cyclohexanes as substrates of acetylcholinesterase and muscarinic agents. A re-investigation, J. Pharm. Pharmacol. 22 (1970) 214–221.
[4] C. Sparr, E. Salamanova, W.B. Schweizer, H.M. Senn, R. Gilmour, Theoretical and X-ray crystallographic evidence of a fluorine-imine gauche effect: an addendum to Dunathan's stereoelectronic hypothesis, Chem. Eur. J. 17 (2011) 8850–8857.
[5] J. Cao, B. Ragnar, M. Bühl, M. Thiel, T. van Mourik, Modelling zwitterions in solution: 3-fluoro-γ-aminobutyric acid (3F-GABA), Chem. Eur. J. 18 (2012) 184–195.
[6] M. Bittencourt, M.P. Freitas, R. Rittner, Conformational and stereoelectronic investigation in 1,2-difluoropropane: the gauche effect, J. Mol. Struct. 840 (2007) 133–136.
[7] L. Goodman, H. Gu, V. Pophristic, Gauche effect in 1,2-difluoroethane. Hyperconjugation, bent bonds, steric repulsion, J. Phys. Chem. A 109 (2005) 1223–1229.
[8] A.G. Deniau, A.M.Z. Slawin, T. Lebl, F. Chorki, J.P. Issberner, T. van Mourik, J.M. Heygate, J.J. Lambert, L.-A. Etherington, K.T. Sillar, D. O'Hagan, Synthesis, conformation and biological evaluation of the enantiomers of 3-fluoro-γ-aminobutyric acid ((R)- and (S)-3F-GABA): an analogue of the neurotransmitter GABA, ChemBioChem 8 (2007) 2265–2274.
[9] I. Yamamoto, G.P. Deniau, N. Gavande, M. Chebib, G.A.R. Johnston, D. O'Hagan, Agonist responses of (R)- and (S)-3-fluoro-γ-aminobutyric acids suggest an enantiomeric fold for GABA binding to $GABA_C$ receptors, Chem. Commun. 47 (2011) 7956–7958.
[10] D.L. Crittenden, M. Chebib, M.J.T. Jordan, Stabilization of zwitterions in solution: γ-aminobutyric acid (GABA), J. Phys. Chem. A 108 (2004) 203–211.
[11] C.T. Walsh, Enzymatic Reaction Mechanisms, 2nd ed., W.H. Freeman, San Francisco, 1979.
[12] J.C. Vederas, H.G. Floss, Stereochemistry of pyridoxal phosphate-catalyzed enzyme reactions, Acc. Chem. Res. 13 (1980) 455–463.
[13] H. Hayashi, H. Wada, T. Yoshimaru, M. Esaki, K. Soda, Recent topics in pyridoxal 5′-phosphate enzyme studies, Annu. Rev. Biochem. 59 (1990) 87–110.
[14] H. Hayashi, Pyridoxal enzymes: mechanistic diversity and uniformity, J. Biochem. 118 (1995) 463–473.
[15] A.C. Eliot, J.F. Kirsch, Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations, Annu. Rev. Biochem. 59 (2004) 383–415.
[16] TDH, Bugg in Introduction to Enzyme and Coenzyme Chemistry, 2nd ed., Wiley-Blackwell, New York, 2004.
[17] H.C. Dunathan, Conformation and reaction specificity in pyridoxal phosphate enzymes, Proc. Natl. Acad. Sci. U S A 55 (1966) 712–716.
[18] R.R. Rando, F.W. Bangerter, Reaction of the neurotoxin gabaculine with pyridoxal phosphate, J. Am. Chem. Soc. 99 (1977) 5141–5145.
[19] M.J. Ondrechen, J.M. Briggs, J.A. McCammon, A model for enzyme-substrate interaction in alanine racemase, J. Am. Chem. Soc. 123 (2001) 2830–2834.
[20] R.B. Silverman, C. George, Inactivation of gamma-aminobutyric acid aminotransferase by (Z)-4-amino-2-fluorobut-2-enoic acid, Biochemistry 27 (1988) 3285–3289.
[21] M. Fu, R.B. Silverman, Isolation and characterization of the product of inactivation of gamma-aminobutyric acid aminotransferase by gabaculine, Bioorg. Med. Chem. 7 (1999) 1581–1590.
[22] R. Casasnovas, A. Salva, J. Frau, J. Donoso, F. Munoz, Theoretical study on the distribution of atomic charges in the Schiff bases of 3-hydroxypyridine-4-aldehyde and alanine. The effect of the protonation state of the pyridine and imine nitrogen atoms, Chem. Phys. 355 (2009) 149–156.
[23] P. Christen, D.E. Metzler, Transaminases, John Wiley & Sons, New York, 1985.
[24] J.N. Jansonius, Structure, evolution and action of vitamin B6-dependent enzymes, Curr. Opin. Struct. Biol. 8 (1998) 759–769.
[25] R.A. John, Pyridoxal phosphate-dependent enzymes, Biochim. Biophys. Acta 1248 (1995) 81–96.
[26] P. Storici, J. Qiu, T. Schirmer, R.B. Silverman, Mechanistic crystallography. Mechanism of inactivation of gamma-aminobutyric acid aminotransferase by (1R,3S,4S)-3-amino-4-fluorocyclopentane-1-carboxylic acid as elucidated by crystallography, Biochemistry 43 (2004) 14057–14063.
[27] R.B. Silverman, M.A. Levy, Mechanism of inactivation of gamma-aminobutyric acid-alpha-ketoglutaric acid aminotransferase by 4-amino-5-halopentanoic acids, Biochemistry 20 (1981) 1197–1203.
[28] R.B. Silverman, B.J. Invergo, Mechanism of inactivation of gamma-aminobutyrate aminotransferase by 4-amino-5-fluoropentanoic acid. First example of an enamine mechanism for a gamma-amino acid with a partition ratio of 0, Biochemistry 25 (1986) 6817–6820.
[29] Z. Wang, H. Yuan, D. Nikolic, R.B. Van Breemen, R.B. Silverman, (±)-(1S,2R,5S)-5-Amino-2-fluorocyclohex-3-enecarboxylic acid. A potent GABA aminotransferase inactivator that irreversibly inhibits via an elimination-aromatization pathway, Biochemistry 45 (2006) 14513–14522.
[30] H. Gökcan, F.A.S. Konuklar, Theoretical study on HF elimination and aromatization mechanisms: a case of pyridoxal 5′ phosphate-dependent enzyme, J. Org. Chem. 77 (2012) 5533–5543.
[31] A. Salva, J. Donoso, J. Frau, F. Munoz, Theoretical studies on Schiff base formation of vitamin B6 analogues, J. Mol. Struct. (Theochem) 577 (2002) 229–238.
[32] A. Salva, J. Donoso, J. Frau, F. Munoz, Theoretical studies on transimination of vitamin B6 analogs, Int. J. Quantum Chem. 89 (2002) 48–56.
[33] A.T. Durak, H. Gökcan, F.A.S. Konuklar, Theoretical studies on the inactivation mechanism of γ-aminobutyric acid aminotransferase, Org. Biomol. Chem. 9 (2011) 5162–5171.
[34] S. Alunni, F. De Angelis, L. Ottavi, M. Papavasileiou, F. Tarantelli, Evidence of a borderline region between E1cb and E2 elimination reaction mechanisms: a combined experimental and theoretical study of systems activated by the pyridine ring, J. Am. Chem. Soc. 127 (2005) 15151–15160.
[35] A.D. Becke, Density-functional thermochemistry. III. The role of exact exchange, J. Chem. Phys. 98 (1993) 5648–5652.
[36] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision A.1, Gaussian, Inc., Wallingford, CT, 2009.

[37] (a) M. Cossi, V. Barone, R. Cammi, J. Tomasi, Ab-initio study of solvated molecules: a new implementation of the polarizable continuum model, J. Chem. Phys. Lett. 255 (1996) 327–335;
(b) V. Barone, M. Cossi, J. Tomasi, Geometry optimization of molecular structures in solution by the polarizable continuum model, J. Comput. Chem. 19 (1998) 404–417;
(c) M. Caricato, F. Ingrosso, B. Mennucci, J. Tomasi, A time-dependent polarizable continuum model: theory and application, J. Chem. Phys. 122 (1999) 154501.
[38] A.E. Reed, L.A. Curtiss, F. Weinhold, Intermolecular interactions from a natural bond orbital. Donor–acceptor viewpoint, Chem. Rev. 88 (1988) 899–926.
[39] R.-Z. Liao, W.-J. Ding, J.-G. Yu, W.-H. Fang, R.-Z. Liu, Water-assisted transamination of glycine and formaldehyde, J. Phys. Chem. A 111 (2007) 3184–3190.
[40] R.D. Bach, C. Canepa, M.N. Glukhovtsev, Influence of electrostatic effects on activation barriers in enzymatic reactions: pyridoxal 5′-phosphate-dependent decarboxylation of a-amino acids, J. Am. Chem. Soc. 121 (1999) 6542–6555.
[41] N.M.F.S.A. Cerqueira, P.A. Fernandes, M.J. Ramos, Computational mechanistic studies addressed to the transimination reaction present in all pyridoxal 5′-phosphate-requiring enzymes, J. Chem. Theory Comput. 7 (2011) 1356–1368.
[42] J. Ortega-Castro, M. Adrover, J. Frau, A. Salvà, J. Donoso, F. Mun˜oz, DFT studies on Schiff base formation of vitamin B6 analogues. Reaction between a pyridoxamine-analogue and carbonyl compounds, J. Phys. Chem. A 114 (2010) 4634–4640.
[43] R.-Z. Liao, W.-J. Ding, J.-G. Yu, W.-H Fang, R.-Z. Liu, Theoretical studies on pyridoxal 5′-phosphate-dependent transamination of alpha-amino acids, J. Comput. Chem. 29 (2008) 1919–1929.
[44] D. Gunajyoti, Zwitterionic conformers of pyrrolysine and their interactions with metal ions – a theoretical study, J. Mol. Model. 19 (2013) 2981–2991.
[45] Z. Huang, Z. Lin, Detailed ab initio studies of the conformers and conformational distributions of gaseous tryptophan, J. Phys. Chem. A 109 (2005) 2656–2659.
[46] C. Ze-qin, Z. Cheng-hua, X. Ying, Theoretical studies on the thermodynamics and kinetics of the N-glycosidic bond cleavage in deoxythymidine glycol, J. Phys. Chem. B 113 (2009) 10409–10420.
[47] M.J. Frisch, M. Head-Gordon, J.A. Pople, A direct mp2 gradient-method, Chem. Phys. Lett. 166 (1990) 275–280.
[48] CYLview, version 1.0b, Université de Sherbrooke, Legault, C.Y., 2009.
[49] I. Humelnicu, E.-U. Würthwein, G. Haufe, The conformers of 3-fluoroalanine. A theoretical study, Org. Biomol. Chem. 10 (2012) 2084–2093.
[50] C.R.S. Briggs, M.J. Allen, D. O'Hagan, D.J. Tozer, A.M.Z. Slawin, A.E. Goeta, J.A.K. Howard, The observation of a large gauche preference when 2-fluoroethylamine and 2-fluoroethanol become protonated, Org. Biomol. Chem. 2 (2004) 732–740.
[51] D.Y. Buissonneaud, T. van Mourik, D. O'Hagan, A DFT study on the origin of the fluorine gauche effect in substituted fluoroethanes, Tetrahedron 66 (2010) 2196–2202.
[52] I. Hyla-Kryspin, S. Grimme, S. Hruschka, G. Haufe, Conformational preferences and basicities of monofluorinated cyclopropyl amines in comparison to cyclopropylamine and 2-fluoroethylamine, Org. Biomol. Chem. 6 (2008) 4167–4175.
[53] J.P. Snyder, N.S. Chandrakumar, H. Sato, D.C. Lankin, The unexpected diaxial orientation of *cis*-3,5-difluoropiperidine in water: a potent CF···NH charge-dipole effect, J. Am. Chem. Soc. 122 (2000) 544–545.
[54] L. Hunter, The C–F bond as a conformational tool in organic and biological chemistry, Beilstein J. Org. Chem. 6 (2010) 38.
[55] E.-M. Tanzer, L.E. Zimmer, W.B. Schweizer, R. Gilmour, Fluorinated organocatalysts for the enantioselective epoxidation of enals: molecular preorganisation by the fluorine-iminium ion gauche effect, Chem. Eur. J. 18 (2012) 11334–11342.
[56] N.E.J. Gooseman, D. O'Hagan, M.J.G. Peach, A.M.Z. Slawin, D.J. Tozer, R.J. Young, An electrostatic gauche effect in β-fluoro- and β-hydroxy-N-ethylpyridinium cations, Angew. Chem. Int. Ed. 46 (2007) 5904–5908.
[57] S. Alunni, V. Laureti, L. Ottavi, R. Ruzziconi, Catalysis of the beta-elimination of HF from isomeric 2-fluoroethylpyridines and 1-methyl-2-fluoroethylpyridinium salts. Proton-activating factors and methyl-activating factors as a mechanistic test to distinguish between concerted E2 and E1cb irreversible mechanisms, J. Org. Chem. 68 (2003) 718–725.
[58] F.D. Angelis, F. Tarantelli, S. Alunni, Ab initio molecular dynamics simulations of elimination reactions in water solution: exploring the borderline region between the E1cb and E2 reaction mechanisms, J. Phys. Chem. B 110 (2006) 11014–11019.
[59] M. Bouclier, M.J. Jung, B. Lippert, Stereochemistry of reactions catalysed by mammalian-brain L-glutamate 1-carboxy-lyase and 4-aminobutyrate: 2-oxoglutarate aminotransferase, Eur. J. Biochem. 98 (1979) 363–368.
[60] A.E. Martell, Chemical and Biological aspects of Vitamin B6 catalysis. Part A, Alan E. Liss Inc., New York, 1984.