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# Molecular modeling of the lipase-catalyzed hydrolysis of acetoxymethyl(*i*-propoxy)phenylphosphine oxide and its P-borane analogue

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ABSTRACT

The molecular modeling of the CAL-B-promoted hydrolysis reactions of acetoxymethyl (*i*-propoxy)phenylphosphine oxide and its P-borane analogue, acetoxymethyl(*i*-propoxy)-phenylphosphine P-borane, confirms that the reactions proceed with the same stereochemistry and in both cases the (*S*)-enantiomers are preferentially transformed by the enzyme. Molecular mechanics calculations show that the main reason for the particular stereoselectivity of the substrates is the steric effect of the phenyl group which causes a remarkable hindrance when placed inside the active site. The replacement of the oxygen by a borane group at the phosphorus stereogenic center does not nullify the stereorecognition by the enzyme, although for the P-borane a lower stereoselectivity is observed. The latter is explained in terms of a smaller energy difference between complexes of CAL-B and particular enantiomers of the P-borane in comparison with those of the phosphine oxide, resulting from the steric effect of the $BH_3$ group. The results helped to revise the previously published erroneous conclusions concerning absolute configuration of the phosphine–borane complex.



## 1. Introduction

Chiral, non-racemic organophosphorus compounds containing a stereogenic phosphorus atom play an important role in various areas of current research, such as asymmetric organic synthesis, biochemistry and catalysis. Among various methods of their synthesis, the one based on the enzyme-promoted stereoselective transformations has become a subject of growing interest [1]. In this way, a series of optically active hydroxymethylphosphine oxides 1, interesting as precursors of herbicides [2], were synthesized by us using either their lipase-promoted acetylation (Scheme 1) or lipase-promoted hydrolysis of the O-acetyl derivatives 2, both performed under kinetic resolution conditions. Their absolute configurations were determined [3,4].

Concerning the applicability of P-chiral phosphorus compounds it must be stressed that trivalent phosphorus compounds, especially tertiary phosphines, are much more interesting since they are used as chiral ligands in transition metal catalysts. Unfortunately, trivalent phosphorus compounds are generally prone to oxidation and usually difficult to handle. Therefore, there is only one example reported in the literature on the enzymatic transformation of hydroxy phosphines [5]. In this context there has recently been a growing interest in the synthesis and transformations of borane complexes of trivalent phosphorus compounds [6–8], since, in contrast to phosphines and other derivatives of trivalent phosphorus, they are stable compounds and can easily be converted into the corresponding $P^{III}$ compounds without racemization. Following this tendency we applied some time ago the biocatalytic methodology for the synthesis of optically active borane analogues of compounds 1, namely P-chiral hydroxymethylphosphine P-boranes 3. Our investigations involved kinetic resolution of racemic P-chiral alkoxy(hydroxymethyl)phenylphosphine P-boranes 3 via their enzymatic acetylation (Scheme 2) or enzymatic hydrolysis of their O-acetyl derivatives 4 [9].

It turned out that the P-boranes 3 were poorer substrates for lipase-catalyzed transformations in comparison with the P-chiral alkoxy(hydroxymethyl)phenylphosphine oxides 1 described previously, and underwent similar reaction much more slowly and with low stereoselectivity. Thus, for the enzymatic acetylation of 1c the enantiomer ratio $E = 32$ [4], while for 3c $E$ is only about 3 [9]. Moreover, the P-borane derivatives, in contrast to the analogous phosphine oxides are not crystalline, which made their X-ray analysis impossible. Therefore, the absolute configuration of enantiomers was ascribed by chemical correlation assuming, by analogy to the borane reduction of bicyclic phosphine oxides [10], that the reaction proceeded with retention of configuration at phosphorus (Scheme 3).

On this basis, the stereochemistry of the lipase-catalyzed acetylation of 3 was considered to be as shown in Scheme 2. Interestingly, comparison of the stereochemical course of the two analogous

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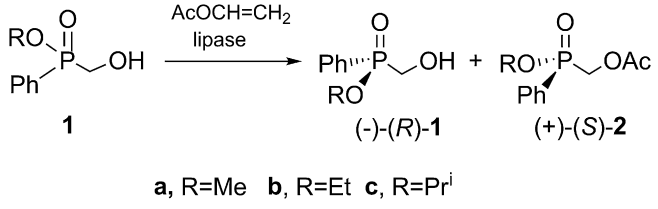
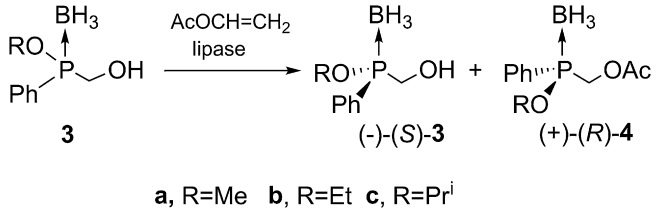
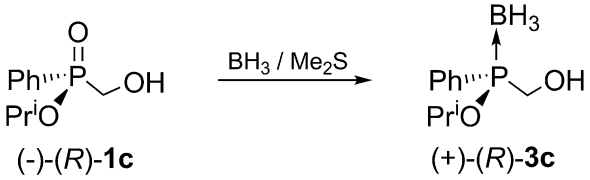



**Scheme 1.** Kinetic resolution of alkoxy(hydroxymethyl)phenylphosphine oxides 1.




**Scheme 2.** Kinetic resolution of alkoxy(hydroxymethyl)phenylphosphine boranes 3.

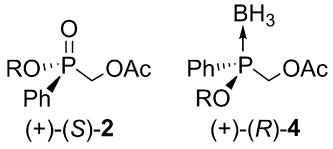



**Scheme 3.** Determination of the absolute configuration of 3 by chemical correlation [9].

enzymatic reactions, acetylation of 1 (Scheme 1) and acetylation of 3 (Scheme 2), indicated that the same enzyme recognized and preferentially transformed the enantiomers of 1 and 3 which had a reverse spatial arrangement around phosphorus. Both preferentially formed enantiomers of 2 and 4 are shown in Scheme 4.

This would mean that a simple replacement of the oxygen atom in the phosphine oxide by the borane moiety in the P-borane would result in the opposite steric course of the enzymatic acetylation reaction. Such a different behavior of enzymes seemed very interesting and prompted us to perform both a more thorough study on chemical correlation and molecular modeling to understand more deeply the mechanism of this process and the surprising reversal of the stereoselectivity upon replacement of O by $BH_3$.

Thus, we have recently reported determination of the absolute configurations of P-boranes 4 based on both extensive DFT calculations and advanced experimental chemical correlation study [11] which contradicts conclusions drawn earlier [9] and points to the configuration of the preferentially transformed enantiomer (+)-4c to be (*S*). Here we present the complementary molecular mechanics (MM) study of CAL-B-promoted hydrolysis of acetoxymethyl(*i*-propoxy)phenylphosphine oxide and its P-borane analogue, acetoxymethyl(*i*-propoxy)-phenylphosphine P-borane. It should be added, that this is the first report dealing with molecular modeling of a lipase-promoted hydrolysis of P-chiral derivatives and only the second concerning a similar operation on heteroatom-chiral compounds, the previous one being devoted to sulfinyl




**Scheme 4.** Absolute configurations of preferentially transformed enantiomers.

derivatives [12]. Computational chemistry offers useful tools that can help in the explanation of both the selectivity and reactivity as well as in the determination of the key factors governing the stereochemistry. Although the MM methods provide rather coarse approximation to a real system, they are computationally inexpensive. Moreover, they have been proven to be helpful in interpretation of similar problems [13]. The conclusions drawn from the present study support our recent results on the stereochemical course of the enzymatic reaction which was proven to be opposite to that shown in Scheme 2 [11].

## 2. Computational methods

All QM (DFT) and MM (Amber) calculations were carried out with the Gaussian 03 package [14] through its graphical interface GaussView 4.1 [15] Optimal geometries of the substrates and model intermediate structures were calculated at the B3LYP/6-31G* level in the gas phase. Using DFT calculations the missing parameter values for the AMBER96 force field [16] have been computed. A modified version of the AMBER force field was used for all energy calculations involving the enzyme.

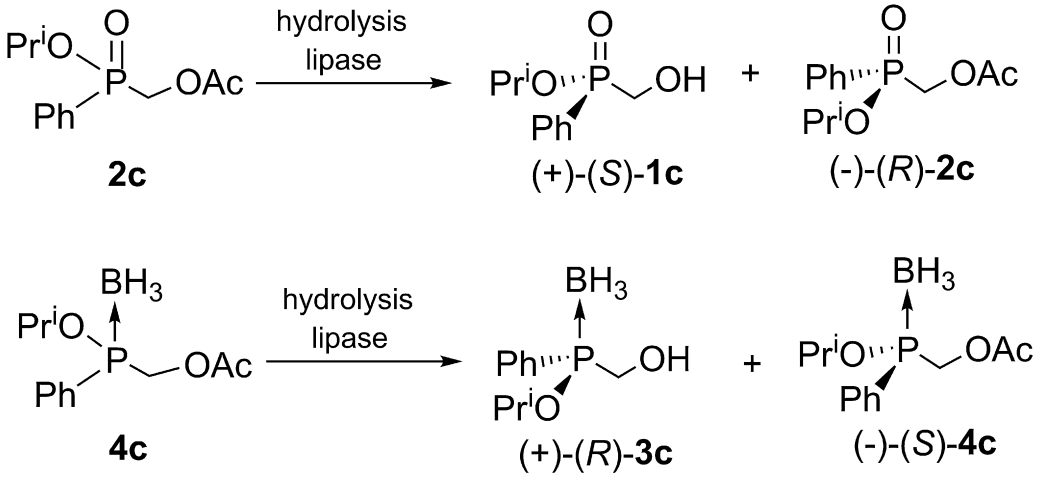
### 2.1. Preparation of starting structures

#### 2.1.1. Preparation of the enzyme starting structure

Structure of the lipase CAL-B was prepared in the following manner. The protein structure used for this study was retrieved from the Protein Data Bank (entry number 1LTB) [17]. The two sugar subunits (NAG318 and NAG319) bound to amide group of Asn74 were removed since being located on the opposite side of the enzyme in respect to the active site, they are believed to have little influence on the selectivity of hydrolysis. The ligand methyl(oxyethyl)heptadecanoate T80 was removed from the active site of the enzyme. We have applied the PropKa program (v. 3.1) [18] for determining the approximate p$K_a$ values for all amino acids in order to add hydrogen atoms to reproduce the protonation state of pH 7. The Asp134 placed on the bottom of the active site has unusual p$K_a$ value of 8.76 suggesting protonation of the carboxyl group. Deeper analysis of the environment of the Asp134 shows that one of the carboxylic oxygen atoms is only 2.64 Å far away from oxygen of the Gln157 proving the presence of the strong hydrogen bond. The catalytic histidine (His224) was defined in the HID AMBER protonation state in order to make it capable of forming the hydrogen bond with Ser105 (the distance between N and O is 2.976 Å which suggests the existence of the hydrogen bond). All water molecules present in the X-ray structure of CAL-B were removed during each optimization.

#### 2.1.2. Preparation of the substrate structures

The original AMBER force field [16] does not include parameters representing the particular interactions for phosphine oxides and their P-borane derivatives; thus in the first step the required force field parameters to describe 2c and 4c have been determined. New atom types for phosphorus, boron and hydrogen atoms in $BH_3$ moiety, marked as P1, B and HB, respectively, were implemented in our calculations. Van der Waals parameters $R$ and $\varepsilon$ for the newly generated atom types were selected by analogy with AMBER standard parameter values. Structures of 2c and 4c were optimized at the B3LYP/6-31G* level in the gas phase and the minimum energy geometries were used as starting points for calculations of the missing parameters. Based on the optimized structures, the equilibrium lengths and angles were determined. In order to compute the force constants for bonding interactions, the bonds and angles were scanned in the vicinity of the equilibrium values using the B3LYP/6-31G* method and then fitted to the




**Scheme 5.** Enzymatic hydrolysis of racemic acetates 2c and 4c selected for molecular modeling.

Hook's curve. The results are shown in Table S1 in Supplementary Information. Default AMBER torsional parameters were used for all torsions involving newly defined atom types. Substrate structures were subjected to conformational search and each conformer was optimized at the B3LYP/6-31G* level to identify the most stable conformers.

Atomic charges were assigned on the basis of B3LYP/6-31G* calculations according to ESP Merz-Kollman scheme [19]. Scheme S1 in Supplementary Information presents the atom types and atomic charges obtained for the model substrates.

### 2.2. Docking protocol

The most abounded conformers of the substrates in terms of free energy values were chosen for docking. Docking was performed with the aid of the AutoDock program version 4.2 [20]. We have assigned the Gasteiger charges to enzyme and substrates. The enzyme structure was kept frozen during the docking procedure. We have defined the grid box of the $60 \times 60 \times 60$ point size each direction centered on the catalytic Ser105. The Lamarckian Genetic Algorithm was applied in this global search. We have defined 100 populations, counting 1000 individuals each, with maximum of generations of 100,000 and sufficient number of energy evaluations. Moreover, we allowed the system to evolve with the crossover ratio = 0.8 and mutation ratio = 0.02. Many structures of the enzyme–ligand complex generated by AutoDock program, were not catalytically active conformations in terms of orientation of the substrate relative to the hydrophilic/hydrophobic pocket of the active site. The best conformers were chosen on the basis of the results of the internal scoring function as well as on the basis of geometrical criteria, namely the distance of the catalytic Ser105 from the carbonyl carbon of the substrate and the correct orientation of the carbonyl oxygen toward the Thr40 and Gly106 that constitute the oxyanion hole. Complexes with the lowest binding energy and catalytically favorable interactions were chosen for further refinement.

### 2.3. Preparation of the tetrahedral intermediates

Best conformations of complex of the substrate with enzyme frozen in its crystallographic structure were used as starting geometries for the tetrahedral intermediates preparation. The covalent bond was created between hydroxyl oxygen of Ser105 and carbonyl carbon atom of the substrate with the initial distance of 2 Å. The Ser105 oxygen atom from primary hydroxyl group upon bond formation changed its type from OH to OS. New covalent bond with the enzyme causes several significant changes in the description of the substrate (in terms of the AMBER force field). We have changed the C type of the carbonyl group into CT which reflects better the tetrahedral environment of the carbon atom. Moreover, we have introduced new atom type Ox for the oxygen atom bearing a formal negative charge. Since the whole fragment: substrate and serine have the overall charge of −1, new point charges were assigned according to the DFT ESP calculations [19]. All new bond angles between OS_Ser105 and substrate CT-Ox, CT-CT and CT-OS were set to 109°. The hydroxyl hydrogen atom of the Ser105 was transferred to His224 upon which the histidine protonation state was changed from HID to HIP. The point charges were adjusted properly to reflect the changes in the geometry. So created structures were optimized in a stepwise manner. All missing parameters involving Ox and the covalent bond with Ser105 were derived from the B3LYP/6-31G* optimized structures of model intermediates shown in Scheme 6. The atom types and partial charges for the tetrahedral intermediates 2c-TI and 4c-TI are presented in Scheme S2 in Supplementary Information.

### 2.4. Optimization procedure

The catalytic complexes generated by AutoDock program were treated in the following manner: the hydrogen positions were optimized, while the coordinates of all heavy atoms were frozen. Such structures were further refined by releasing the limitations for the substrate inside the enzyme with frozen heavy atoms. The last step was releasing the hydrogens, substrate and catalytically-essential amino acids: His224, Ser105 and Thr40. Similar three-step scheme was applied for the tetrahedral intermediates. The values placed in the table correspond to the last step of optimization.

## 3. Results and discussion

Among the candidates tested experimentally [9], the lipase from *Candida antarctica* CAL-B was chosen for modeling study because it was found to show the best stereoselectivity in the systems studied, and its X-ray structure is known. *Candida antarctica* lipase B (CAL-B) is an efficient catalyst for hydrolysis of ester substrates in water and esterification in organic solvents [21,22]. CAL-B consists of 317 amino acid residues. The most important part of CAL-B responsible for its activity is the catalytic triad consisting of Ser105, His224, and Asp187 [23,24]. In contrast to most lipases, CAL-B has no lid that shields the active site, but the hydrophobic substrate-binding site is solvent exposed [25].

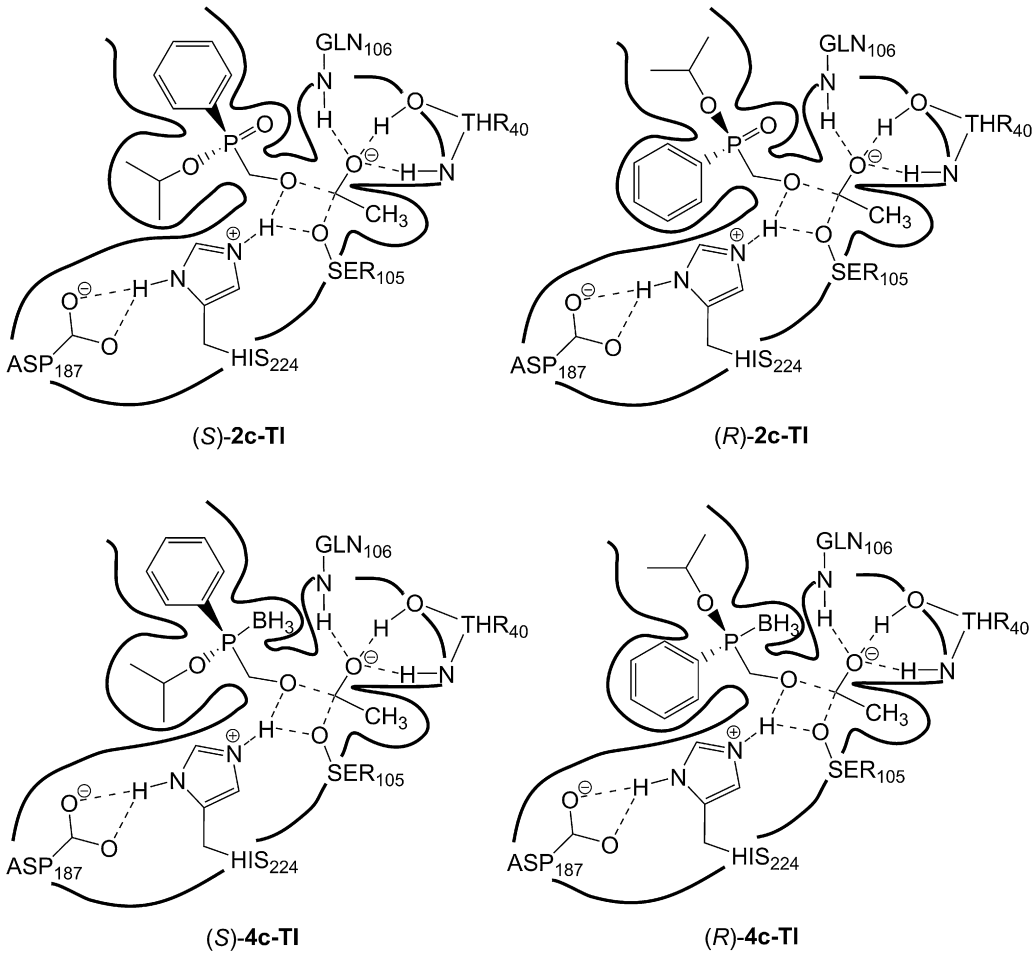



**Fig. 1.** Schematic representations of four productive geometries for the tetrahedral intermediates 2c-TI and 4c-TI inside the active pocket of CAL-B; the catalytically essential hydrogen bonds are marked with dashed lines.

### 3.1. Interaction of substrates with the enzyme

Since enzymatic hydrolysis of 2 and 4 and acetylation of 1 and 3 are the reverse processes, intermediates and transition states in both reactions must be the same and both reactions must proceed with the same stereochemistry. The hydrolysis reaction was selected for calculations for the convenience reasons. First, docking of the acetyl derivatives 2c and 4c in the enzyme active site is easier as it requires manipulation of only one molecule of the substrate instead of the two independent molecules. Second, the complexes and intermediates formed during the acetylation reaction involve the acetylated form of CAL-B which is not available in the PDB database. Thus, the transformations, which will be considered, are as shown in Scheme 5.

As model substrates for theoretical studies of enzymatic hydrolysis, we have chosen both enantiomers of acetoxymethyl(i-propoxy)phenylphosphine oxide (2c) and its P-borane analogue (4c) (Schemes 1 and 2). Geometries of the substrates were optimized by the DFT method (B3LYP/6-31G(d)) and the conformational search was performed to select the lowest in energy conformers. Than the most stable conformers were docked into the active site of CAL-B by means of the Autodock 4.2 program [20]. All calculations involving enzyme have been performed using the modified AMBER96 force field [16]. The missing force field parameters for the substrates have been estimated from DFT calculations. For each substrate, the conformation presenting the highest score and fulfilling the structural requirements for the initiation of the enzymatic reaction, was selected for further modeling steps.

### 3.2. Tetrahedral intermediates (transition state analogues)

We have used the protocol that has been successfully applied to rationalize the selectivity of the enzyme-aided hydrolysis of esters [26–36]. The enantioselectivity of the hydrolysis stems from the difference in the potential energy between the substrates and the corresponding transition state. Since true transition states for enzymatic reactions are difficult to find, we have used the tetrahedral intermediates resulting from the attack of serine on the carbonyl C atom in the acetoxy group as an approximation of the transition state. This is a frequently applied procedure and the tetrahedral intermediates are usually considered a reasonable model of the transition states [12,26,30,37].

Tetrahedral intermediates (TI) are formed as a result of the attack of serine on the carbonyl C atom in 2c and 4c. To construct TI's we used optimized structures of complexes, in which the substrates were manually linked to Ser105 and allowed for partial optimization (for details see Section 2). To find the missing AMBER parameters for the serine-substrate linkage, the serine-imitating fragment has been attached to the substrate bond as shown in Scheme 6 and optimized by the DFT method (Scheme 6).

We have taken into consideration only the catalytically productive structures, i.e., those conformations that do not show any steric clashes and in which all six catalytically active hydrogen bonds were present (see Fig. 1) [30]. Upon formation of the covalent bond with Ser105, the negative charge is built up on carbonyl oxygen of the acetyl group which results in formation of catalytically active hydrogen bonds with Thr40, Gln106 and His224 (Fig. 1).

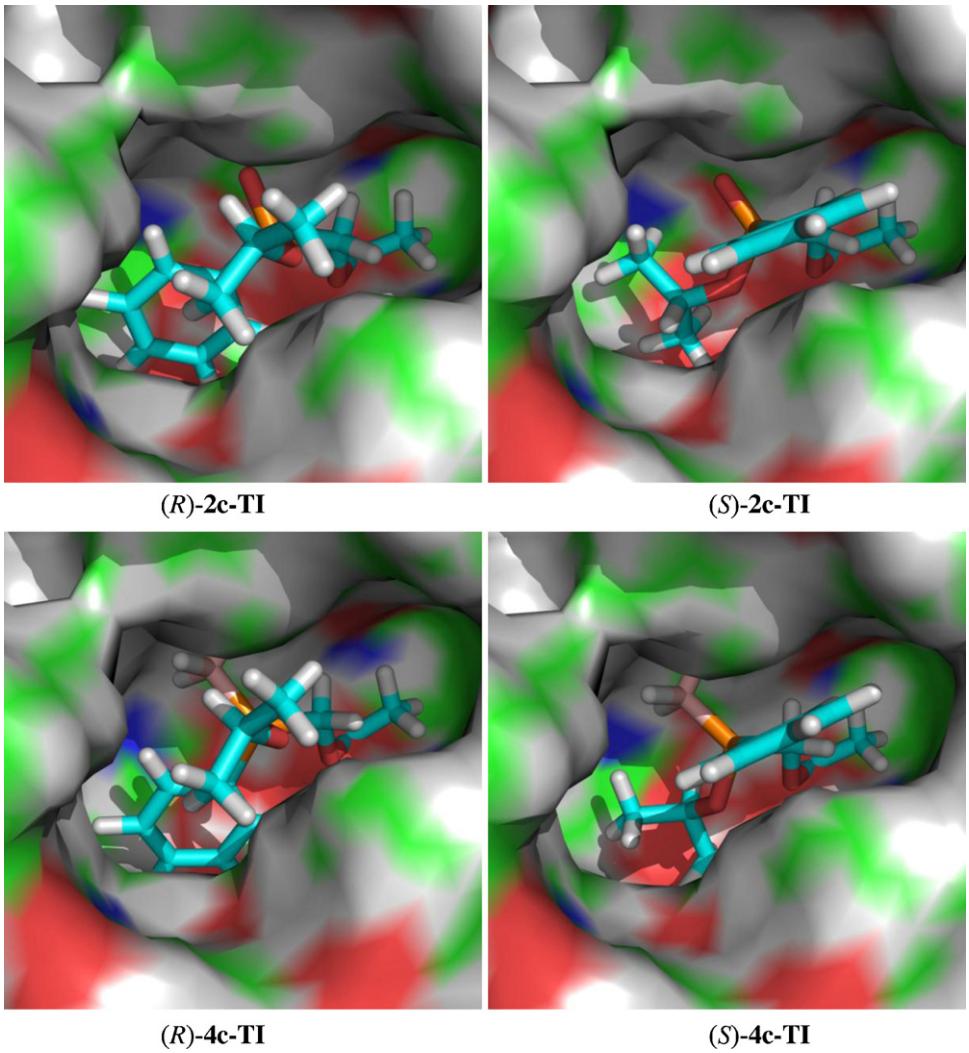
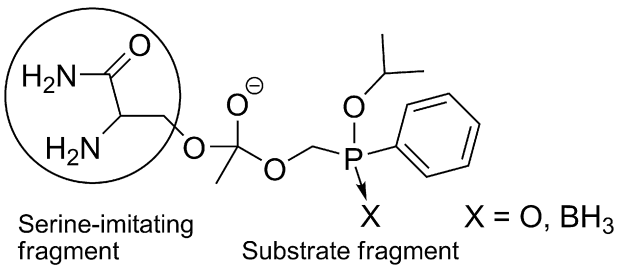



**Fig. 2.** Catalytically productive tetrahedral intermediates for the CAL-B-catalyzed hydrolysis of (*R*)- and (*S*)-enantiomers of 2c and 4c, as identified by molecular modeling. The important active site and substrate atoms (sticks) are colored as follows: cyan (carbon), white (hydrogen), red (oxygen), orange (phosphorus), and pink (boron). Surrounding atoms (space fill) of the enzyme are shown in grey.

As a consequence of these interactions, the substrate fragment moves deeper into the active pocket. Two best arrangements for each enantiomer were examined: the first one having the phenyl ring localized in the entrance pocket of the active site and the second one with the isopropoxy moiety occupying this pocket. The third possible conformation, with P=O or P-$BH_3$ moieties pointing towards solvent, was not obtained due to unfavorable steric interactions when both bulky substituents, Ph and $OPr^i$, were oriented towards the interior of the enzyme. The lowest energy structures of all four tetrahedral intermediates are given in Supplementary Information. The arrangements of the substrates within the active site is schematically shown in Fig. 1. It should be noted that the present projection in Fig. 1 is not simply analogous to that published in Ref. [29] since a different viewpoint has been applied here for a better clarity.




**Scheme 6.** Model complex structures resulting from the attack of serine-analogue on the carboxylic carbon atom in 2c and 4c.

In the lowest energy geometry of (*S*)-2c-TI the phenyl ring is placed in the entrance pocket and $OPr^i$ is located inside the enzyme in the hydrophobic pocket. The acetyl group is fixed in the nearest vicinity of the catalytic triad. The negatively charged oxygen atom (Ox) occupies the oxyanion hole and is involved in three hydrogen bonds, namely HO(Thr40)-Ox(lig), H(Thr40)-Ox(lig) and H(Gln106)-Ox(lig) (Fig. 1). Methyl group does not induce any steric conflict as the closest contact with the neighboring amino acids is 2.10 Å (Gly39). The phenyl ring occupies the entrance pocket and the $OPr^i$ group fits into the inner pocket with the closest nonbonding H···H distance of 2.28 Å. The P=O group is surrounded by Val155 and Gln157. Despite the high polarity of the P=O bond, the phosphoryl oxygen atom does not form any hydrogen bond with the nearest polar protons (see Supplementary Information for the pdb structures of the tetrahedral intermediates).

In the tetrahedral intermediate (*R*)-2c-TI the P=O and acetoxy groups take positions very similar to those found in (*S*)-2c-TI. The

**Table 1**
The absolute energies (in h) of the tetrahedral intermediates and energy differences between (*S*)- and (*R*)-enantiomers (in kcal/mol).

| | | (*R*)-2c | (*S*)-2c | (*R*)-4c | (*S*)-4c |
|---|---|---|---|---|---|
| Complexes | h (kcal/mol) | −12.13645 (5.4) | −12.14503 (0) | −12.03733 (3.9) | −12.04049 (0) |
| Intermediates | h (kcal/mol) | −12.11928 (14.4) | −12.14215 (0) | −12.07838 (0.9) | −12.07980 (0) |

bulky substituents at phosphorus are interchanged: the phenyl ring is located inside the enzyme, and $OPr^i$ remains in the entrance pocket. The phenyl group suffers from unfavorable short-range interatomic interactions with the surrounding amino acid fragments. The shortest nonbonding distance of phenyl hydrogens to amino acid hydrogens are 2.01 Å (Thr138), 1.99 Å (Val190), 1.97 Å (Gln157). The bulkiness of the phenyl ring placed inside the enzyme causes the shortening of the (Ser105)O···HN(His224) bond. On the other hand, the isopropoxyl group occupying the entrance pocket is in close contact to Ile189 (2.11 Å and 2.21 Å) and Ile285 (2.23 Å).

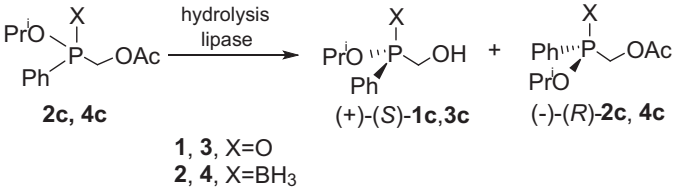
The lowest energy geometries of the tetrahedral intermediates 4c-TI show similar orientation of the substituents as in the case of 2c-TI. The (*S*)-4c-TI places the phenyl ring into the entrance pocket. The planar phenyl ring fits well into the pocket without any close repelling interactions. Most of the nonbonding interatomic distances between Ph ring and the surrounding amino acid fragments are longer than 3 Å. $BH_3$ group is accommodated between Gln157, Thr40 and Val154. The distances from H(B) to Gln157 and Val154 are in the range of 1.99 to 2.60 Å, which indicates that the larger $BH_3$ moiety can induce sterically unfavorable contacts, which may explain why P-borane analogues 4c are less effectively recognized by the CAL-B. The $OPr^i$ directed to the center of the active site does not reveal any significant steric hindrance (the closest contact to Gln157 and Val190 are 2.15 Å and 2.22 Å, respectively; other interatomic distances are greater than 2.5 Å).

The optimal orientation of (*R*)-4c-TI is analogous to (*R*)-2c-TI with $OPr^i$ located in the entrance pocket and Ph pointing to the interior of the active site. As in the case of (*R*)-2c-TI, the phenyl ring inside the pocket induces some steric hindrance (distances 1.95 Å with Val190, 2.18 Å with Ile189 and 2.26 Å with hydrogens of Gln157). The $BH_3$ group closely encounters the surrounding amino acid fragments Ile285 and Thr40 (H···H nonbonding distances ranging from 1.90 Å to 1.98 Å). Optimized structures of catalytically productive tetrahedral intermediates are shown in Fig. 2.

Comparison of the energies of the enantiomers of tetrahedral intermediates for P═O and P-borane substrates reveals that in both cases the (*S*) enantiomers are more energetically favored. Phosphine oxide (*S*)-2c-TI intermediate is by 14.4 kcal/mol lower in energy than its (*R*) analogue. This result is in accord with the kinetic studies which proved that the (*S*)-2c was the fast-reacting enantiomer [3,4]. The (*S*)-4c-TI intermediate is more stable than the (*R*)-4c-TI by about 0.9 kcal/mol which is in accord with our current interpretation of the experimental results [11], and contradictory to the previous paper [9] stating that the fast-reacting enantiomer of 4c is that of (*R*) configuration. The results of calculations are presented in Table 1.

The deeper insight into the interatomic distances between enzyme and the substrate suggests that the main reason for the fact that (*R*)-2c is more weakly bound to the active site of CAL-B than (*S*)-2c are unfavorable steric interactions between Ph and the active pocket. The steric hindrance of the borane group may also contribute to this effect. Moreover, small energy difference between enantiomers of 4c-TI intermediates compared to 2c-TI corresponds well with the observed much lower selectivity of the reaction with P-borane 4c.

It should be stressed that in the case of the phosphine oxide 2c, the result of the molecular modeling, based on the theoretical calculations of the energy of complexes of particular enantiomers




**Scheme 7.** The true stereochemical course of the enzymatic hydrolysis of 2 and 4.

with CAL-B, is in full agreement with the earlier experimental finding, i.e., that the (*S*)-enantiomer is preferentially recognized and transformed by the enzyme. The case of 4c was controversial since the absolute configuration of this ester has not been directly (e.g., by X-ray) determined so far [9,11]. Nevertheless, current investigations strongly support that it is the (*S*)-enantiomer of 4c, whose complex with CAL-B has lower energy and therefore it should be preferentially recognized and transformed by the enzyme. Such a result strongly suggests that the absolute configuration of (−)-3c and (+)-4c must have been originally erroneously ascribed [9]. This has been ultimately confirmed by the reinvestigation of the stereochemical course of the reaction of borane with 1c leading to 3c with inversion of configuration at phosphorus [11] (thus, Scheme 3 shows the incorrect stereochemistry). Hence, the molecular modeling indicates that the stereochemical course of both reactions shown in Scheme 5 is the same (Scheme 7).

Theoretical calculations presented above have clearly proven to be useful in predicting the stereochemical course of enzyme-promoted reactions. Moreover, in this case they allowed to verify the wrong conclusions which were drawn earlier on the basis of an incorrect determination of the absolute configuration of the reaction products [9].

## 4. Conclusions

The simple molecular modeling, based on the molecular mechanics (Amber96) calculations of the structures and energies of intermediates of particular enantiomers with CAL-B, shows that the enzymatic hydrolysis reactions of acetoxymethyl(*i*-propoxy)phenylphosphine oxide and its P-borane analogue, acetoxymethyl(*i*-propoxy)phenylphosphine P-borane, proceed with the same stereochemistry and in both cases the (*S*)-enantiomers are preferentially transformed by the enzyme. Although a low-cost, approximate approach was used, the results support our experimental findings. Calculations show that the main reason for this particular stereoselectivity in both P-chiral phosphorus esters considered is the steric effect of the phenyl group which causes a remarkable hindrance when placed inside the active site. The replacement of the oxygen by a larger borane group at the phosphorus stereogenic center induces additional steric hindrance which, consequently, causes a decrease in energy difference between the enantiomers of tetrahedral intermediates of P-borane with CAL-B compared to those involving the phosphine oxide. The smaller energy difference in the case of P-borane enantiomers corresponds to a lower stereoselectivity of the enzyme, which is indeed observed for this substrate. The above results allowed to correct the previously published erroneous conclusions concerning absolute configuration of the phosphine–borane complex which has been recently verified experimentally.

## Supplementary Information

Structures of tetrahedral intermediates (in pdb format) and the force field parameters for the substrates 2c and 4c are given.

## Acknowledgements

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## 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.2012.09.001.

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