Supporting Information for:

Expanding the Catalytic Triad in Epoxide Hydrolases and Related Enzymes

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S1. Calibrating the Relevant Reference Reactions for EVB Simulations

S1.1. Calibrating the first step of the reaction: Nucleophilic attack

Epoxide hydrolysis is an extensively studied reaction, and these compounds are known to hydrolyze spontaneously\(^1\), or through acid\(^1\)\(^{-}\)\(^8\) or base\(^1\)\(^9\) catalysis. The challenge here is the fact that in our system, nucleophilic attack of D105 leads to the formation of an oxyanion, which is likely to be a hugely unfavorable reaction at ambient pH. In fact, no direct experimental data are available to characterize this step of the reaction; the closest available experimental data correspond to the reaction between propylene oxide and acetate as an anion in acidic conditions (glacial acetic acid), for which an activation barrier of 18.5–19.0 kcal·mol\(^{-1}\) has been reported\(^2\). However, the fact that the reaction considered in ref. \(^2\) is acid-catalyzed means that this value only provides a lower limit for the activation barrier and is not a real estimate of the activation barrier for the uncatalyzed reaction modeled here. In the absence of direct experimental data for this process, we have estimated the energetics of this step using DFT calculations of the model reaction. Bruice et al. have previously studied the uncatalyzed reaction of methyl styrene oxide with acetate as a nucleophile\(^4\). For this reaction, they obtained a gas phase activation barrier of 20.0 and 21.0 kcal·mol\(^{-1}\) for C1 and C2 attack, respectively. However, when solvation effects were included (at the CPCM+B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) level of theory), the activation barrier increased to 29.9 and 32.5 kcal·mol\(^{-1}\) for C1 and C2 attack, respectively. Here, we have performed an unconstrained transition state optimization using trans-stilbene oxide and propionate anion as a model. The geometry optimization for this system was performed using the B3LYP functional\(^{10-12}\) and the 6-311+G** basis set. Solvation was implicitly included using the C-PCM continuum solvent model\(^{13}\) with the UFF radii. The resulting transition state was characterized by frequency calculations, as well as by following the intrinsic reaction coordinate.
(IRC)\textsuperscript{14,15} to minima in both reactant and product directions. Entropy and zero-point energy contributions were obtained by performing frequency calculation on the stationary points (Table S9). All quantum chemical calculations were performed using Gaussian 09\textsuperscript{16}.

It should be noted that the choice of the B3LYP functional as our reference state was deliberate and we are aware of the errors that could affect this estimation, which in some cases also cancel out by the use of this functional\textsuperscript{17}. For example, it is well known that the description of anionic species is extremely challenging with DFT methods, as they tend towards unphysical charge delocalization\textsuperscript{18}. This problem, combined with the poor solvation of anionic species by implicit solvent models, can lead to artificially low reaction barriers. This has been well established when using hydroxide and other anionic species as a nucleophile in calculations with implicit solvation\textsuperscript{19}. In previous studies we introduced a constant correction for the undersolvation of the hydroxide ion to reproduce the experimental solvation free energy of this anion\textsuperscript{19}. Similar corrections have also been suggested by others\textsuperscript{20}. However, in the present case it is unclear what the appropriate correction factor would be, and therefore in light of the (at least partial) accumulation of errors between not including dispersion and inappropriate solvation of the charged species, we believe it is safer to use a functional that is not corrected for dispersion in order to avoid remedying one error while maintaining another, thus potentially obtaining completely unphysical results. Additionally, using B3LYP allows us to directly compare to previous work\textsuperscript{21-23}. Despite these challenges, in the context of the current work, it is again crucial to emphasize that here the reference reaction in aqueous solution is only used as a frame of reference to compare the energetics of the reaction with different histidine protonation states (Table S3) and to facilitate direct comparison with previous works in related systems that use this functional\textsuperscript{21,22,24}. Our results show that we can reproduce the large expected
catalytic effect of StEH1 and the trends in activation barrier upon mutation (see Tables S5 and S6 and Figure 8 in the main text). These results in themselves provide a rigorous test of the reliability of our overall parameterization and calibration procedure.

As the substrate is symmetrical, the overall activation barrier for the nucleophilic attack on either C1 or C2 would presumably be the same for both enantiomers in solution. However due to small differences in orientation of the epoxide ring relative to the acetate nucleophile at the transition state, the activation energy could vary slightly. Following the protocol described at the beginning of this section, we calculated an activation barrier of 32 kcal·mol$^{-1}$ and a reaction free energy of 22 kcal·mol$^{-1}$ for the uncatalyzed alkylation step in solution (see Figure 1 of the main text for an overview of the catalytic cycle). These values are similar to those previously reported by Bruice et al. for the nucleophilic attack of acetate on methyl styrene oxide$^4$. While the activation barrier may seem quite high, opening the epoxide ring to yield a charged oxyanion, is highly unfavorable in aqueous solution, and, in fact, the experimental values for acid-catalyzed or spontaneous epoxide ring-opening to give a protonated oxygen are much lower$^2$. Furthermore, as can be seen in the main text, the catalytic effect of the enzyme on this step is quite large, as the oxyanion is stabilized by not one but two residues forming the oxyanion hole, Y154 and Y235, and the oxygen of the D105 is stabilized by the backbone amides of F33 and W106. The effect of tyrosine has been experimentally$^{25,26}$ and theoretically$^{4,27}$ investigated using phenol and 1,8-bis-phenylenediol as tyrosine models. Bruice et al. have shown that general-acid catalysis by phenol can decrease the activation energy by 10 kcal·mol$^{-1}$ compared to the uncatalyzed reaction$^4$. However, it has been suggested that the stabilization provided by the two tyrosines, even though is an important contributor to catalysis, is not as great as that exhibited by 1,8-bis-phenylenediol in butanone solvent$^4$. 
S1.2. **Calibrating the second step: hydrolysis of the alkyl-enzyme intermediate**

The reference reaction for the second step (hydrolysis of the alkyl-enzyme intermediate) is far more straightforward to calibrate, as both acid- and base-catalyzed ester hydrolysis were studied experimentally in detail. Experimental data\(^{28}\) for imidazole as general base suggest an activation barrier of about 19 kcal·mol\(^{-1}\) (correcting also for the cost of bringing the reacting fragments from infinite separation into the reacting complex, which is our starting point for the calculations here – see refs. \(^{29,30}\). The reaction free energy can be derived from experiments investigating the breakdown of analogs of tetrahedral intermediate species and oxygen exchange data for ester hydrolysis reactions\(^{31}\). Here, the free energy is related to the activation energy for the breakdown reaction as:

\[
k_{\text{reverse}} = \left(\frac{k_e}{k_h}\right) \cdot 2k_{\text{breakdown}}
\]

where \(k_e\) is the pseudo-first order rate constant for oxygen exchange with water and \(k_h\) is the first order rate constant for general-based catalyzed hydrolysis of water at the carbonyl carbon. The values of the ratio \(k_e/k_h\) have been reported by Bender and Heck\(^{31}\), with values varying between 0.15 to 0.7 for different substrates. The rate for the breakdown has been estimated to be approximately \(10^7\) s\(^{-1}\) for structural analogues\(^{32}\). From these values, the free-energy barrier for the reverse reaction was estimated as 8.6 kcal·mol\(^{-1}\), leading to a free energy of \(\Delta G_{\text{INT}2} = 10.4\) kcal·mol\(^{-1}\). Therefore, \(\Delta G_{\text{INT}2} = 19\) kcal·mol\(^{-1}\) and \(\Delta G_{\text{INT}2} = 10\) kcal·mol\(^{-1}\) were used as approximations to calibrate the reaction where histidine acts as a base. Note here that as we do not model the final breakdown of the tetrahedral intermediate, and we are examining a range of mutants relative to the wild-type enzyme (i.e. we are interested in the relative rather than absolute values), the precise free energy for the formation of this intermediate is less crucial. As mentioned above, for the final step of the cycle (**Figure 1** of the main text), it is unclear whether
the reaction proceeds through an actual tetrahedral intermediate or not, and what the lifetime of such a reaction would be, but this has limited impact on our computational studies, as it is known to be very fast and not rate-limiting. The only feature of potential interest here is the fact that the anionic oxygen after opening the epoxide ring will likely be protonated at some point upon breakdown of the tetrahedral intermediate; however, this is very challenging to model computationally within a VB framework, and is, again, not a contributor to the rate-limiting step of the reaction.
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S3. Figures & Tables

**Figure S1**: Active-site structures with corresponding σ-weighted \((2m|Fo| − D|Fc|)^{33}\) electron density maps, for (A) wild-type, and (B) H300N mutant forms of StEH1. Maps are contoured at their RMSD values (0.29 e/Å\(^3\) for the wild-type, and 0.38 e/Å\(^3\) for the mutant). Note that the electron density for the hydrolytic water (shown between D105 and E35 in panel A) is lost in the H300N mutant structure.
**Figure S2:** Residue frequency calculated based on sequence alignments of: (A) all members of the epoxide hydrolase-like superfamily showing the aspartate nucleophile, (B) enzymes from the epoxide hydrolase subfamily that have the aspartate nucleophile and (C) members of the dehalogenase enzyme family showing the aspartate nucleophile. The corresponding plots from aligning BLAST results to the StEH1 sequence and selecting annotated sequences from mammals, plants, fungi and bacteria can be found in Figure 5 of the main text. The plots show the probability of finding a given residue at a particular sequence position, with the *Solanum tuberosum* epoxide hydrolase residue numbers indicated below the sequence. H104 has high sequence conservation in all cases, with the background of unrelated residues in the sequence database likely to be caused by either unrelated or mischaracterized sequences in the database, as the results starting from a BLAST search show almost total conservation. In this search, only members of the dehalogenase family show a different residue, glutamine, at this position.
**Figure S3:** Overlay of representative transition state (TS) structures for the hydrolytic step of the reaction catalysed by StEH1. Shown here are TS obtained from water attack on (A) the intermediate formed after attack at the C1 carbon of TSO, and (B) the intermediate formed after attack at the C2 carbon of TSO. Here, the (R,R)-TSO intermediates are shown in red, and the (S,S)-TSO intermediates are shown in blue.
Figure S4: Active site of the (A) wild-type and (B) E35Q variants of StEH1 at the tetrahedral intermediate formed upon alkylation of the (R,R) (blue) and (S,S) (yellow) enantiomers of TSO (blue). This figure is based on structures obtained from the last EVB-FEP/US window of our simulations (corresponding to the tetrahedral intermediate formed from hydrolysis of the alkyl-enzyme intermediate, see Figure 1 of the main text). The panels are shown in two parts: the ball-and-stick structures show a single snapshot from the endpoint of our EVB simulations, while the cluster of conformers shown in line format are the different E35 and Q35 conformations from our EVB simulations of this reaction step. In the wild-type variant (panel A), E35 points towards (protonated) H104 forming a salt-bridge. In the E35Q variant (panel B), Q35 preferentially interacts with either the hydrolytic water molecule ((S,S)-enantiomer, yellow), or is much more
flexible, and points away from the substrate complex and out of the active site \(((R,R)\text{-enantiomer, blue})\). In the latter case, this allows great solvent accessibility to the reacting atoms, whereas the E35-H104 and Q35-hydrolytic water interactions act as a closed “gate”, preventing solvent access to the active site. Note that the views at the top and bottom of each panel are identical structures that have been rotated to better illustrate the differences in the position of residue 35 in the different systems.
Table S1: Data collection and refinement statistics. The space group is P2₁,2₁,2₁. Information in parentheses refers to the highest resolution shell.

| Data collection statistics                                                                 |
|-------------------------------------------------------------------------------------------|
| Data collection beamline/detector                                                          | ESRF ID14:2/ACDS scanner                                                                 |
| Cell axial lengths (Å)                                                                     | 56.0, 96.0, 121.6                                                                         |
| Resolution range (Å)                                                                      | 75.81 - 2.0 (2.05 - 2.00)                                                                  |
| Number of reflections measured                                                             | 206,332                                                                                    |
| Number of unique reflections                                                               | 45,161                                                                                     |
| Average multiplicity                                                                      | 4.6 (4.6)                                                                                  |
| Completeness (%)                                                                          | 100.0 (100.0)                                                                              |
| Rmeas (%)                                                                                 | 8.0 (34.1)                                                                                 |
| σ                                                                                        | 14.7 (4.4)                                                                                 |
| Wilson B-factor (Å²)                                                                       | 20.1                                                                                       |

| Refinement statistics                                                                      |
|-------------------------------------------------------------------------------------------|
| Resolution range (Å)                                                                      | 75.38 - 2.0                                                                                |
| Number of reflections used in working set                                                  | 42,817                                                                                    |
| No. of reflections for R_free calculation                                                  | 2276                                                                                      |
| R-value, R_free (%)                                                                       | 14.6, 19.8                                                                                |
| No. of non-hydrogen atoms                                                                 | 5632                                                                                      |
| No. of solvent waters                                                                     | 521                                                                                       |
| Mean B-factor, protein atoms, A and B molecules (Å²)                                      | 21.5, 17.2                                                                                |
| Mean B-factor, solvent atoms (Å²)                                                         | 32.0                                                                                      |
| Ramachandran plot outliers (%)<sup>a</sup>                                                | 6 (1%)                                                                                    |
| r.m.s. deviation from ideal bond length (Å)<sup>b</sup>                                   | 0.018                                                                                     |
| r.m.s. deviation from ideal bond angle (°)<sup>b</sup>                                    | 1.801                                                                                     |

<sup>a</sup> Calculated using a strict-boundary Ramachandran plot<sup>34</sup>.  
<sup>b</sup> Using the parameters of Engh & Huber<sup>35</sup>.  

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**Table S2:** Comparison of residues at position 35 and 104 (StEH1 numbering), indicating residue conservation to preserve charge neutrality in the active site. Sequences presented here were obtained from BLAST as described in the main text. The green cells indicate conservation of the H104-E/D35 pattern at the active site, while the red cells indicate replacement of H104 by another residue. As can be seen, when H104 is replaced by a non-ionizable residue, the adjacent E35 is replaced by either a Ser or Thr residue.

| #  | Seq. ID          | Residue Number | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 101 | 102 | 103 | 104 | 105 | 106 | 107 |
|----|-----------------|----------------|----|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|
| 1  | tr|Q41415|Q41415_SOLTU | G  | F  | P  | E  | L  | W  | Y  | V  | V  | A  | H  | D  | W  | G  |
| 2  | tr|S8E221|S8E221_9LAM1 | G  | F  | P  | E  | L  | W  | Y  | V  | V  | G  | H  | D  | W  | G  |
| 3  | tr|D8L7V7|D8L7V7_PRUPE | G  | F  | P  | E  | L  | W  | Y  | V  | V  | G  | H  | D  | W  | G  |
| 4  | tr|B9RT84|B9RT84_RICCO | G  | F  | P  | E  | L  | W  | Y  | V  | V  | G  | H  | D  | W  | G  |
| 5  | tr|W9RQ4|W9RQ4_9ROSAS | G  | F  | P  | E  | L  | W  | Y  | V  | V  | G  | H  | D  | W  | G  |
| 6  | tr|D8L7V8|D8L7V8_PRUPE | G  | F  | P  | E  | L  | W  | Y  | L  | V  | G  | H  | D  | W  | G  |
| 7  | tr|Q76E1|Q76E1_CITJA  | G  | F  | P  | E  | L  | W  | Y  | L  | V  | G  | H  | D  | W  | G  |
| 8  | tr|B9SAT9|B9SAT9_RICCO | G  | F  | P  | E  | L  | W  | Y  | L  | V  | G  | H  | D  | W  | G  |
| 9  | tr|Q39856|Q39856_SOYBN | G  | F  | P  | E  | L  | W  | Y  | L  | V  | A  | H  | D  | W  | G  |
| 10 | tr|O49857|O49857_SOYBN | G  | F  | P  | E  | L  | W  | Y  | L  | V  | A  | H  | D  | W  | G  |
| 11 | tr|B9RF7|B9RF7_RICCO | G  | F  | P  | D  | L  | W  | Y  | L  | V  | G  | H  | D  | W  | G  |
| 12 | tr|B3VRM3|B3VRM3_NICBE | G  | F  | P  | E  | L  | W  | Y  | L  | V  | A  | H  | D  | W  | G  |
| 13 | tr|B9GSC2|B9GSC2_POPTR | G  | F  | P  | E  | L  | W  | Y  | L  | V  | G  | H  | D  | W  | G  |
| 14 | tr|A9PBN3|A9PBN3_POPTR | G  | F  | P  | E  | L  | W  | Y  | L  | V  | G  | H  | D  | W  | G  |
| 15 | tr|A2Q30|A2Q30_MEDTR  | G  | F  | P  | E  | L  | W  | Y  | L  | V  | A  | H  | D  | W  | G  |
| 16 | tr|B9I7B7|B9I7B7_POPTR | G  | F  | P  | E  | L  | W  | Y  | L  | V  | A  | H  | D  | W  | G  |
| 17 | tr|B9RF7|B9RF7_RICCO | G  | F  | P  | E  | L  | W  | Y  | L  | V  | A  | H  | D  | W  | G  |
| 18 | tr|B3VMR4|B3VMR4_NICBE | G  | F  | P  | E  | L  | W  | Y  | L  | V  | A  | H  | D  | W  | G  |
| 19 | tr|G7KX11|G7KX11_MEDTR | G  | F  | P  | E  | L  | W  | Y  | L  | V  | A  | H  | D  | W  | G  |
| 20 | tr|I2CW84|I2CW84_MACMU | G  | F  | P  | E  | S  | W  | Y  | F  | I  | G  | H  | D  | W  | G  |
| 21 | tr|Q17QK4|Q17QK4_BOVIN | G  | F  | P  | E  | S  | W  | F  | F  | I  | G  | H  | D  | W  | G  |
| 22 | tr|S9Q9A4|S9Q9A4_PTEAL | G  | F  | P  | E  | S  | W  | F  | F  | I  | G  | H  | D  | W  | G  |
| 23 | tr|F1RJ53|F1RJ53_PIG   | G  | F  | P  | E  | S  | W  | F  | F  | I  | G  | H  | D  | W  | G  |
| 24 | sp|Q6Q2C2|HYES_PIG    | G  | F  | P  | E  | S  | W  | F  | F  | I  | G  | H  | D  | W  | G  |
| 25 | tr|L8H3|L8H3_9CEPA  | G  | F  | P  | E  | S  | W  | F  | I  | G  | H  | D  | W  | G  | G  |
| 26 | tr|K7CY76|K7CY76_PANTR | G  | F  | P  | E  | S  | W  | F  | I  | G  | H  | D  | W  | G  | G  |
| 27 | sp|P34913|HYES_HUMAN  | G  | F  | P  | E  | S  | W  | F  | I  | G  | H  | D  | W  | G  | G  |
| 28 | tr|U3F8Z4|U3F8Z4_CALJA | G  | F  | P  | E  | S  | W  | F  | I  | G  | H  | D  | W  | G  | G  |
| 29 | tr|F7FNE8|F7FNE8_CALJA | G  | F  | P  | E  | S  | W  | F  | I  | G  | H  | D  | W  | G  | G  |
| 30 | tr|K9IAL5|K9IAL5_DESRO | G  | F  | P  | E  | S  | W  | F  | F  | I  | G  | H  | D  | W  | G  |
| Accession     | Expression | Function | Tissue | Development | Pathway | Disease | Mutation |
|--------------|------------|----------|--------|-------------|---------|---------|----------|
| M1EL05       |            |          |        |             |         |         |          |
| G5BY92       |            |          |        |             |         |         |          |
| Q5RKK3       |            |          |        |             |         |         |          |
| S7PCN0       |            |          |        |             |         |         |          |
| G7PZN4       |            |          |        |             |         |         |          |
| L8I2A0       |            |          |        |             |         |         |          |
| U3Auw4       |            |          |        |             |         |         |          |
| L7JMW7       |            |          |        |             |         |         |          |
| Q3UB32       |            |          |        |             |         |         |          |
| J81MCJ1      |            |          |        |             |         |         |          |
| W9C033       |            |          |        |             |         |         |          |
| K1W7D7       |            |          |        |             |         |         |          |
| M7T034       |            |          |        |             |         |         |          |
| F2RN91       |            |          |        |             |         |         |          |
| V5HR52       |            |          |        |             |         |         |          |
| B2WHB7       |            |          |        |             |         |         |          |
## Members of the Dehalogenase Enzyme Family using an Aspartate Nucleophile

| #  | Seq. ID                  | Residue Number |
|----|-------------------------|----------------|
| 72 | gi|34922418|sp|OS2866.3-HYES_CORS2 | G W P Q S W Y V I G H D W G |
| 73 | gi|34922418|sp|OS2866.3-DHAA_RILHO | G N P T S S H L V A Q D W G |
| 74 | gi|306755666|sp|Q1JU72.1-DEHA_BURSP | G F P Q N L H L V G H D R G |
| 75 | gi|28558082|sp|P59337.1-DHAA_BRADU | G N P T S S H L V A Q D W G |
| 76 | gi|461925|sp|Q01398.1-DEH1_MORSB | G F P Q N R A L V G H D R G |
| 77 | gi|28558081|sp|P59336.1-DHAA_RHOSD | G N P T S S Y L V I H D W G |
| 78 | gi|6122634|sp|A5U5S9.1-DHAA_MYCTA | G N P T S S Y L V I H D W G |
| 79 | gi|9789853|sp|P51698.4-LINB_SPHPI | G N P T S S Y L V I H D W G |
| 80 | gi|28558089|sp|Q93K00.1-DHAA_MYCAV | G E P T W S Y L F V Q D W G |
| 81 | gi|28558089|sp|Q8U671.1-DHAA_AGRTP | G N P T S S Y L V L Q D Y G |
| 82 | gi|167016874|sp|Q93K00.1-DHAA_MYCAV | G E P T W S Y L F V Q D W G |
| 83 | gi|226729402|sp|B2HJU9.1-DHAA_RHORH | G N P T S S Y L V I H D W G |
| 84 | gi|226729403|sp|B4RF90.1-DHAA_RHORH | G N P T S S Y L V I H D W G |
| 85 | gi|28558089|sp|Q8U671.1-DHAA_AGRTP | G E P T W S Y L F V Q D W G |
| 86 | gi|54036966|sp|P64302.1-DHMA1_MYCBO | G E P T W S Y L F V Q D W G |
| 87 | gi|122415385|sp|Q1QBB9.1-DHMA_PSYCK | G E P T W S Y L F V Q D W G |
| 88 | gi|189083120|sp|B0SY51.1-DHMA_CAUSK | G E P T W S Y L F V Q D W G |
| 89 | gi|54036967|sp|P64304.1-DHMA2_MYCBO | G N P T W S F S M G Q D W G |
| 90 | gi|729681|sp|P22643.2-DHMA_XANAU | G E P T W S Y L V V Q D W G |
| 91 | gi|152805199|sp|Q938B4.1-DHAA_MYSX2 | G N P T S S Y L V I H D W G |
| 92 | gi|81829712|sp|Q6NAM1.1-DHAA_RHOPA | G F P Q T H V L A G H D R G |
**Table S3:** Calculated\(^{(a)}\) and observed\(^{(b)}\) activation (\(\Delta G^\ddagger\)) and reaction free energies (\(\Delta G^\circ\)), in kcal\(\cdot\)mol\(^{-1}\), for the hydrolysis of (\(R,R\))- and (\(S,S\))-TSO for the wild-type enzyme and different H104 protonation states. HIP and HID refer to the protonated and deprotonated forms of H104, following the nomenclature used by the force field. The corresponding EVB parameters are presented in Section S4.

| System   | Step I     |               | Step II    |               |
|----------|------------|---------------|------------|---------------|
|          | \(\Delta G_1^\ddagger\) | \(\Delta G_1^\circ\) | \(\Delta G_2^\ddagger\) | \(\Delta G_2^\circ\) | \(\Delta G_4^{\text{ktat}}\) |
|          | Calc. | Exp. | Calc. | Exp. | Calc. | Exp. | Calc. | Exp. |
| (\(R,R\))-TSO |
| C1 HID    | 13.0±1.7 | 14.4 | -8.7±1.9 | -1.7 | 6.2±1.9 | 15.8 | -6.3±2.1 | 15.9 |
| C2 HID    | 11.6±1.3 | 14.4 | -8.1±1.3 | -1.7 | 3.3±1.3 | 15.8 | -8.4±1.5 | 15.9 |
| C1 HIP    | 18.3±1.1 | 14.4 | 3.4±1.6  | -1.7 | 24.8±2.4 | 15.8 | 16.6±2.6 | 15.9 |
| C2 HIP    | 14.7±1.0 | 14.4 | 0.2±1.4  | -1.7 | 17.7±1.6 | 15.8 | 8.6±1.7  | 15.9 |
| (\(S,S\))-TSO |
| C1 HID    | 12.1±2.1 | 16.0 | -6.2±2.6 | -0.2 | 9.1±2.2 | 17.0 | -2.5±2.3 | 16.9 |
| C2 HID    | 13.6±2.1 | 16.0 | -8.7±2.2 | -0.2 | 3.8±2.2 | 17.0 | -8.2±2.2 | 16.9 |
| C1 HIP    | 15.2±0.6 | 16.0 | 2.2±1.0  | -0.2 | 24.0±2.3 | 17.0 | 16.6±2.6 | 16.9 |
| C2 HIP    | 16.9±0.8 | 16.0 | 0.4±1.1  | -0.2 | 16.6±1.7 | 17.0 | 8.8±1.8  | 16.9 |

\(^{(a)}\) All calculated energies are averages and standard deviations based on ten individual EVB simulations generated from different starting structures, as outlined in the Methodology section. \(\Delta G_1^\ddagger\) and \(\Delta G_2^\ddagger\) correspond to activation barriers for the alkylation and hydrolysis steps respectively, with the barrier to the hydrolysis step corrected by adding the calculated activation barrier of the hydrolysis step to the free energy of the intermediate. For the corresponding uncorrected (absolute) activation barriers, see Table S4. \(^{(b)}\) Exp. refers to experimental values of the enzyme-catalyzed reaction for the respective step, derived from the kinetic data presented in ref. 36–39.
Table S4: Uncorrected (absolute) activation free energies, in kcal·mol⁻¹, for the hydrolysis step of the (R,R)- and (S,S)-TSO reactions in wild-type StEH1⁴,⁵.

| System | (R,R)-TSO | (S,S)-TSO |
|--------|-----------|-----------|
|        | ΔG⁺₂ | ΔG⁺₀ | ΔG⁻₂ | ΔG⁻₀ | ΔG⁺₂ | ΔG⁺₀ |
|        | Calc. | Exp.  | Calc. | Exp.  | Calc. | Exp.  |
| C1 HID | 14.9±0.2 | 15.8 | 2.5±0.9 | 15.3±1.5 | 17.0 | 3.8±1.5 |
| C2 HID | 11.3±0.4 | 15.8 | -0.4±0.8 | 12.5±0.8 | 17.0 | 0.5±1.1 |
| C1 HIP | 21.4±1.6 | 15.8 | 13.3±1.8 | 21.8±1.6 | 17.0 | 14.3±2.1 |
| C2 HIP | 17.6±0.4 | 15.8 | 8.4±0.4 | 16.2±1.1 | 17.0 | 8.4±1.3 |

(a) All calculated energies are averages and standard deviations based on ten individual EVB simulations generated from different starting structures, as outlined in the Methodology section. ΔG⁺₂ corresponds to activation barriers for the hydrolysis step. (b) Exp. refers to experimental values of the enzyme-catalyzed reaction for the respective step, respectively, derived from the kinetic data presented in ref. 36-39.

Table S5: Calculated⁴ and observed⁵ activation energies (ΔG⁺) and reaction free energies (ΔG⁰), in kcal·mol⁻¹, for the hydrolysis of (R,R)-TSO for different mutant forms of StEH1. The corresponding EVB parameters are presented in Section S4.

| System | Step I |  |  | Step II |  |  |  |
|--------|--------|--------|--------|--------|--------|--------|--------|
|        | ΔG⁺₂   | ΔG⁺₀   | ΔG⁻₂   | ΔG⁻₀   | ΔG⁺₂   | ΔG⁺₀   | ΔG⁺₁kJcat |
|        | Calc.  | Exp.   | Calc.  | Exp.   | Calc.  | Exp.   | Calc.   |
| C1     |        |        |        |        |        |        |        |
| E35Q   | 18.5±1.1 | 16.1±1.5 | -0.5±1.1 | 21.2±1.6 | 17.8±1.5 | 9.2±1.5 | 18.1    |
| Y149F  | 14.8±1.1 | 14.3±1.5 | -1.8±1.1 | 17.9±1.2 | 15.3±1.5 | 9.7±1.4 | 15.5    |
| Y154F  | 18.7±0.8 | n.d.   | 4.1±1.0 | n.d.   | 21.4±1.7 | n.d.   | 12.4±2.1 | 19.3    |
| Y235F  | 17.8±0.7 | n.d.   | 5.4±1.2 | n.d.   | 29.2±1.0 | n.d.   | 21.8±1.0 | 19.3    |
| H300N  | 17.5±1.0 | 18.5±1.0 | -0.3±1.1 | n.d.   | n.d.   | n.d.   | n.d.    |

C2

| E35Q   | 15.5±0.7 | 16.1±1.3 | -0.5±1.3 | 17.4±3.1 | 17.8±3.3 | 6.2±3.3 | 18.1    |
| Y149F  | 13.0±0.5 | 14.3±1.5 | -3.1±0.9 | 13.5±4.3 | 15.3±7.4 | 7.4±3.9 | 15.5    |
| Y154F  | 16.2±1.3 | n.d.   | 2.9±1.5 | n.d.   | 22.3±1.7 | n.d.   | 14.5±1.6 | 19.3    |
| Y235F  | 18.4±1.3 | n.d.   | 5.7±1.5 | n.d.   | 21.3±2.1 | n.d.   | 12.0±2.3 | 19.3    |
| H300N  | 16.8±0.8 | 18.5±1.0 | 1.9±0.9 | n.d.   | n.d.   | n.d.   | n.d.    |

(a) All calculated energies are averages and standard deviations based on ten individual EVB simulations generated from different starting structures, as outlined in the Methodology section. ΔG⁺₁ and ΔG⁻₁ correspond to activation barriers for the alkylation and hydrolysis steps respectively, with the barrier to the hydrolysis step corrected by adding the calculated EVB barrier to the free energy of the intermediate from the hydrolysis step. For the corresponding uncorrected (absolute) activation barriers, see Table S7. (b) Exp. refers to experimental values of the enzyme-catalyzed reaction for the respective step, derived from the kinetic data presented in ref. 36-39. n.d.: Not determined. (c) In most cases H104 is protonated and D265 is deprotonated, the exceptions to this are the reactions involving the H300N and E35Q mutants, where H104 is most likely neutral. Additionally, in the H300N variant D265 is protonated, as discussed in the main text.
**Table S6:** Calculated\(^{(a)}\) and observed\(^{(b)}\) activation energies (\(\Delta G^\ddagger\)) and reaction free energies (\(\Delta G^\circ\)), in kcal·mol\(^{-1}\), for the hydrolysis of \((S,S)\)-TSO for different mutant forms of StEH1. The corresponding EVB parameters are presented in Section S4.

| System | Step I |          |          |          |          |          |          |          |
|--------|--------|----------|----------|----------|----------|----------|----------|----------|
|        | \(\Delta G^\ddagger_1\) | \(\Delta G^\circ_1\) | \(\Delta G^\ddagger_2\) | \(\Delta G^\circ_2\) | \(\Delta G^\ddagger_{kcat}\) |          |          |          |
|        | Calc.  | Exp.     | Calc.    | Exp.     | Calc.    | Exp.     | Calc.    | Exp.     |
| C1     |        |          |          |          |          |          |          |          |
| E35Q   | 17.0±0.8 | 15.6   | 2.2±1.2 | -1.2    | 19.9±1.5 | 17.6   | 8.9±1.6 | 17.6    |
| Y149F  | 15.2±0.4 | 15.5   | 2.0±1.4 | 0.0     | 20.1±1.5 | 16.2   | 12.4±1.5 | 16.6    |
| Y154F  | 16.3±1.6 | n.d.   | 1.0±1.9 | n.d.    | 19.9±2.2 | n.d.  | 12.4±2.2 | n.d.    |
| Y235F  | 17.7±0.8 | n.d.   | 3.0±0.9 | n.d.    | 24.2±0.9 | n.d.  | 16.3±0.9 | n.d.    |
| H300N\(^{(c)}\) | 17.5±0.7 | 19.8   | 2.5±0.8 | -1.2    | n.d.     | n.d.  | n.d.     | n.d.    |
| C2     |        |          |          |          |          |          |          |          |
| E35Q   | 19.3±0.7 | 15.6   | 4.7±0.7 | -1.2    | 20.9±1.5 | 17.6   | 10.8±2.0 | 17.6    |
| Y149F  | 17.1±0.6 | 15.5   | -0.3±0.8 | 0.0     | 15.5±1.0 | 16.2   | 7.4±0.9 | 16.6    |
| Y154F  | 17.6±1.0 | n.d.   | 1.1±1.0 | n.d.    | 16.8±1.4 | n.d.  | 9.3±1.5 | n.d.    |
| Y235F  | 17.6±0.5 | n.d.   | 3.3±0.6 | n.d.    | 20.7±1.1 | n.d.  | 12.1±1.2 | n.d.    |
| H300N\(^{(c)}\) | 21.2±0.4 | 19.8   | 4.9±0.6 | -1.2    | n.d.     | n.d.  | n.d.     | n.d.    |

\(^{(a)}\)All calculated energies are averages and standard deviations based on ten individual EVB simulations generated from different starting structures, as outlined in the Methodology section. \(\Delta G^\ddagger_1\) and \(\Delta G^\ddagger_2\) correspond to activation barriers for the alkylation and hydrolysis steps respectively, with the barrier to the hydrolysis step corrected by adding the calculated activation barrier of the hydrolysis step to the free energy of the intermediate. For the corresponding uncorrected (absolute) activation barriers, see Table S7. \(^{(b)}\) Exp. refers to experimental values of the enzyme-catalyzed reaction for the respective step, derived from the kinetic data presented in ref. \(^{36-39}\). n.d.: Not determined. \(^{(c)}\) In most cases H104 is protonated and D265 is deprotonated, the exceptions to this are the reactions involving the H300N and E35Q mutants, where H104 is most likely neutral. Additionally, in the H300N variant D265 is protonated, as discussed in the main text.
Table S7: Uncorrected (absolute) activation free energies, in kcal·mol⁻¹, for the hydrolysis step of the reaction of various mutant forms of StEH1 with (R,R)- and (S,S)-TSO (a,b).

| System | (R,R)-TSO |  | (S,S)-TSO |  |
|--------|-----------|---|-----------|---|
|        | ΔG²⁺ | ΔG²° | ΔG²⁺ | ΔG²° |
|        | Calc. | Exp. | Calc. | Exp. | Calc. | Exp. | Calc. | Exp. |
| C1     |       |       |       |       |       |       |       |       |
| E35Q   | 17.6±0.8 | 17.8 | 5.6±1.5 | 17.7±0.6 | 17.6 | 6.6±0.8 |
| Y149F  | 19.7±0.5 | 15.3 | 11.5±0.9 | 18.1±0.5 | 16.2 | 10.4±0.6 |
| Y154F  | 17.3±1.3 | n.d. | 8.3±1.8 | 18.9±0.5 | n.d. | 11.4±0.6 |
| Y235F  | 23.9±0.3 | n.d. | 16.4±0.5 | 21.2±0.4 | n.d. | 13.3±0.4 |
| C2     |       |       |       |       |       |       |       |       |
| E35Q   | 16.8±2.4 | 17.8 | 5.6±2.7 | 16.2±1.2 | 17.6 | 6.1±1.7 |
| Y149F  | 16.5±0.3 | 15.3 | 8.7±0.4 | 15.7±0.6 | 16.2 | 7.7±0.8 |
| Y154F  | 19.3±0.4 | n.d. | 11.5±0.5 | 15.7±0.5 | n.d. | 8.2±0.6 |
| Y235F  | 15.6±1.1 | n.d. | 6.3±1.4 | 17.4±0.8 | n.d. | 8.9±0.9 |

(a) All calculated energies are averages and standard deviations based on ten individual EVB simulations generated from different starting structures, as outlined in the Methodology section. ΔG²⁺ corresponds to activation barriers for the hydrolysis step. (b) Exp. refers to experimental values of the enzyme-catalyzed reaction for the respective step, derived from the kinetic data presented in ref. 36-39. n.d.: Not determined.

Table S8: Microscopic rate and thermodynamic constants for StEH1-catalyzed (R,R)-TSO hydrolysis.

| Enzyme     | Kₛ (µM) | k₁ (s⁻¹) | k₂ (s⁻¹) | k₋₂ (s⁻¹) | k₃ (s⁻¹) |
|------------|---------|-----------|-----------|-----------|-----------|
| WT         | 36±22(a) | 260±56(a) | 16±18(a)  | 24±3(b)   |           |
| E35Q       | 27±22(a) | 16±3(a)   | 6.7±2(a)  | 0.95±0.04(a) |           |
| H300N      | 60±40(b) | 0.3±0.06(b) | 0.13±0.02(b) | 0.003±0.0005(b) |           |
| E35Q/H300N | 31±6 | 0.27±0.02 | 0.0083±0.0038 | 0 |           |

(a) Adapted from ref. 38. (b) Adapted from ref. 36.
Table S9: Absolute electronic energy ($E_{el}$, in atomic units), zero-point energy contribution ($E_{ZPE}$, in kcal·mol$^{-1}$), entropies ($S$, in cal·mol$^{-1}$·K$^{-1}$) and frequencies ($\nu$, in cm$^{-1}$) for each optimized stationary point along the reaction profiles of nucleophilic attack of acetate on styrene oxide. RS, TS, and PS denote reactant, transition and product states, respectively.

| Species | $E_{el}$ (a.u) | $E_{ZPE}$ (kcal·mol$^{-1}$) | $S$ (cal·mol$^{-1}$·K$^{-1}$) | $\nu$ (cm$^{-1}$) |
|---------|----------------|-----------------------------|-----------------------------|------------------|
| RS      | -844.779505481 | 167.6949                    | 167.433                     | 1.8              |
| TS      | -844.741593384 | 167.5307                    | 140.424                     | -358.1           |
| PS      | -844.760935349 | 169.1559                    | 138.552                     | 27.3             |

$\Delta G^\ddagger_{calc}$ $^{(a)}$=31.7

$\Delta G^o_{calc}$ $^{(a)}$=21.7

$^{(a)}$ $\Delta G^\ddagger_{calc}$ and $\Delta G^o_{calc}$ refer to the activation and reaction free energies calculated relative to the reactant complex.
S4. Empirical Valence Bond Parameters Used in This Work

The EVB parameters used in this work were obtained as outlined in the Methodology section of the main text. All parameters not listed here are standard OPLS-AA parameters for the relevant atom types.

The off-diagonal elements, describing the coupling between the two diabatic states, can be represented by simple exponential functions of the form:

$$H_{ij} = A_{ij} \exp \left[ -\mu (r_{ij} - r_0) \right]$$

(2)

where $r_{ij}$ denotes the distance between atoms $i$ and $j$ and $r_0$ denotes the equilibrium distance or, as in this work, by constant functions where the parameter $\mu$ is set equal to zero. The off-diagonal elements ($H_{ij}$) and gas-phase shift ($\alpha_i$) values used in this work to calibrate the reference reactions are presented below. For more details of the meaning of these parameters, please see e.g. refs. 40,41. For details of the mechanism please see Figure 1 of the main text. Note that in line with the EVB philosophy, the same parameters were then used unchanged in all enzyme runs (i.e. all fitting was done only in the background reaction, and not in subsequent runs).

Table S10: EVB mapping parameters used in this work\(^{(a)}\).

|                  | \begin{tabular}{l|l|l|l|l} \hline Step I (Alkylation) & \multicolumn{2}{c|}{C1} & \multicolumn{2}{c}{C2} \\ \hline \multicolumn{1}{c|}{(R,R)-TSO} & $H_{ij}$ & $\alpha_i$ & $H_{ij}$ & $\alpha_i$ \\ \hline (S,S)-TSO & 63.84 & 11.04 & 63.87 & 2.37 \\ \hline Step II (Hydrolysis) & \multicolumn{2}{c|}{C1} & \multicolumn{2}{c}{C2} \\ \hline \multicolumn{1}{c|}{(R,R)-TSO} & $H_{ij}$ & $\alpha_i$ & $H_{ij}$ & $\alpha_i$ \\ \hline (S,S)-TSO & 46.49 & 289.19 & 44.61 & 290.29 \\ \hline \end{tabular} | (a) Both $H_{ij}$ and $\alpha_i$ are constants. All values given in kcal mol\(^{-1}\). |
Figure S5: Structures of the different VB states used in this work.
## EVB Parameters

### Table S11: Van der Waals Parameters Used for Atoms Constituting the Reacting Part.

| Type  | \( A_i \) (kcal\(^{1/2}\)·mol\(^{-1/2}\)·Å\(^6\)) | \( B_i \) (kcal\(^{1/2}\)·mol\(^{-1/2}\)·Å\(^3\)) | \( C_i \) (kcal·mol\(^{-1}\)) | \( \alpha_i \) (Å\(^2\)) | \( A_{1-4} \) (kcal\(^{1/2}\)·mol\(^{-1/2}\)·Å\(^6\)) | \( B_{1-4} \) (kcal\(^{1/2}\)·mol\(^{-1/2}\)·Å\(^3\)) | mass (a.u.) |
|-------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------|
| CA    | 1059.13         | 23.67           | 0               | 0               | 748.92          | 16.74           | 12.01    |
| CO4   | 1802.24         | 34.18           | 250             | 1.7             | 1274.38         | 24.17           | 12.01    |
| CT    | 944.52          | 22.03           | 250             | 2.5             | 667.88          | 15.58           | 12.01    |
| CHMI  | 944.52          | 22.03           | 250             | 1.7             | 667.88          | 15.58           | 12.01    |
| H     | 0.00            | 0.00            | 30              | 2.8             | 0.00            | 0.00            | 1.01     |
| HA    | 69.58           | 4.91            | 0               | 0               | 49.20           | 3.47            | 1.01     |
| HC    | 49.20           | 3.47            | 0               | 0.0             | 34.79           | 2.45            | 1.01     |
| HC1   | 109.18          | 6.99            | 0               | 0.0             | 77.20           | 4.94            | 1.01     |
| HC2   | 84.57           | 5.41            | 0               | 0.0             | 59.80           | 3.83            | 1.01     |
| NA    | 971.75          | 28.31           | 180             | 2.5             | 687.13          | 20.02           | 14.01    |
| O     | 616.44          | 23.77           | 90              | 2.2             | 435.89          | 16.81           | 16.00    |
| OCRB  | 616.44          | 23.77           | 90              | 1.8             | 435.89          | 16.81           | 16.00    |
| OH    | 976.93          | 31.26           | 90              | 2.0             | 690.79          | 22.10           | 16.00    |
| OH1   | 690.37          | 23.36           | 0               | 0.0             | 488.17          | 16.87           | 16.00    |
| OH2   | 760.65          | 25.04           | 100             | 2.8             | 537.86          | 17.71           | 16.00    |
| OS    | 445.13          | 18.25           | 90              | 2.0             | 314.75          | 12.91           | 16.00    |
| OS1   | 445.13          | 18.25           | 0               | 0.0             | 314.75          | 12.91           | 16.00    |
| OT3   | 762.88          | 24.39           | 100             | 2.8             | 539.44          | 17.25           | 16.00    |

For all atoms except reacting atoms, a standard 6-12 Lennard Jones potential was used. In the case of the reacting atoms, which change bonding patterns between atoms \( i \) and \( j \), an alternate function of the form: \( V_{\text{react}} = C_i C_j \exp(-\alpha_i \alpha_j r_{ij}) \) was used to prevent artificial repulsion between these atoms as bonding patterns change. \( r_{ij} \) denotes the distance (Å) between atoms \( i \) and \( j \).
Table S12: Atom Types in Different VB States.

| Atom Number | State I | State II | State III | State II | State III |
|-------------|---------|----------|-----------|----------|-----------|
| 1           | CA      | CA       | CA        | CA       | CA        |
| 2           | HA      | HA       | HA        | HA       | HA        |
| 3           | CA      | CA       | CA        | CA       | CA        |
| 4           | HA      | HA       | HA        | HA       | HA        |
| 5           | CA      | CA       | CA        | CA       | CA        |
| 6           | HA      | HA       | HA        | HA       | HA        |
| 7           | CA      | CA       | CA        | CA       | CA        |
| 8           | HA      | HA       | HA        | HA       | HA        |
| 9           | CA      | CA       | CA        | CA       | CA        |
| 10          | HA      | HA       | HA        | HA       | HA        |
| 11          | CA      | CA       | CA        | CA       | CA        |
| 12          | CT      | CT       | CT        | CT       | CT        |
| 13          | HC2     | HC       | HC2       | HC1      | HC1       |
| 14          | OS1     | OH       | OH        | OH       | OH        |
| 15          | CT      | CT       | CT        | CT       | CT        |
| 16          | HC2     | HC1      | HC1       | HC       | HC2       |
| 17          | CA      | CA       | CA        | CA       | CA        |
| 18          | CA      | CA       | CA        | CA       | CA        |
| 19          | HA      | HA       | HA        | HA       | HA        |
| 20          | CA      | CA       | CA        | CA       | CA        |
| 21          | HA      | HA       | HA        | HA       | HA        |
| 22          | CA      | CA       | CA        | CA       | CA        |
| 23          | HA      | HA       | HA        | HA       | HA        |
| 24          | CA      | CA       | CA        | CA       | CA        |
| 25          | HA      | HA       | HA        | HA       | HA        |
| 26          | CA      | CA       | CA        | CA       | CA        |
| 27          | HA      | HA       | HA        | HA       | HA        |
| 28          | CT      | CT       | CT        | CT       | CT        |
| 29          | HC2     | HC       | HC2       | HC       | HC2       |
| 30          | HC2     | HC       | HC2       | HC       | HC2       |
| 31          | CO4     | CO4      | CHM1      | CO4      | CHM1      |
| 32          | OCRB    | OS       | OS1       | OS       | OS1       |
| 33          | O       | O        | OH        | O        | OH        |
| 34          | OT3     | OT3      | OH2       | OT3      | OH2       |
| 35          | H       | H        | H         | H        | H         |
| 36          | H       | H        | H         | H        | H         |
| 37          | CT      | CT       | CT        | CT       | CT        |
| 38          | HC2     | HC2      | HC2       | HC2      | HC2       |
| 39          | HC2     | HC2      | HC2       | HC2      | HC2       |
| 40          | CA      | CA       | CA        | CA       | CA        |
| 41          | NA      | NA       | NA        | NA       | NA        |
|   | H | H | H | H | H | H |
|---|---|---|---|---|---|---|
| 42 | CA | CA | CA | CA | CA | CA |
| 43 | HA | HA | HA | HA | HA | HA |
| 44 | NA | NA | NA | NA | NA | NA |
| 45 | CA | CA | CA | CA | CA | CA |
| 46 | HA | HA | HA | HA | HA | HA |
| 47 | HA | HA | HA | HA | HA | HA |
| #  | (S,S)-TSO C1 Attack | (R,R)-TSO C1 Attack | (S,S)-TSO C2 Attack | (R,R)-TSO C2 Attack |
|----|---------------------|---------------------|---------------------|---------------------|
| 1  | -0.1554             | -0.1381             | -0.1864             | -0.1591             |
| 2  | -0.1465             | -0.1881             | -0.1902             | -0.2195             |
| 3  | 0.1448              | 0.1166              | 0.0918              | 0.1069              |
| 4  | -0.1542             | -0.1176             | -0.1410             | -0.0332             |
| 5  | -0.1465             | -0.1881             | -0.1902             | -0.2195             |
| 6  | 0.1448              | 0.1166              | 0.0918              | 0.1069              |
| 7  | -0.1542             | -0.1176             | -0.1410             | -0.0332             |
| 8  | 0.1279              | 0.0889              | 0.0802              | 0.0597              |
| 9  | -0.1277             | -0.0128             | 0.0894              | -0.1930             |
| 10 | 0.0709              | 0.3399              | 0.2444              | 0.6359              |
| 11 | 0.1485              | 0.0333              | -0.0183             | -0.1122             |
| 12 | -0.2516             | -0.8686             | -0.9634             | -0.8867             |
| 13 | 0.0709              | 0.5376              | 0.5850              | -0.1615             |
| 14 | 0.1485              | -0.1447             | -0.1801             | 0.0439              |
| 15 | 0.1277              | -0.0714             | -0.0554             | -0.0739             |
| 16 | -0.1542             | -0.0684             | -0.0543             | 0.0003              |
| 17 | 0.1229              | 0.0894              | 0.0892              | 0.0811              |
| 18 | -0.1277             | -0.0128             | 0.0894              | -0.1930             |
| 19 | 0.1485              | 0.1078              | 0.1006              | 0.1261              |
| 20 | -0.1542             | -0.0684             | -0.0543             | 0.0003              |
| 21 | 0.1229              | 0.0894              | 0.0892              | 0.0811              |
| 22 | -0.1465             | -0.1837             | -0.2373             | -0.2299             |
| 23 | 0.1448              | 0.1078              | 0.1006              | 0.1261              |
| 24 | -0.1542             | -0.0684             | -0.0543             | 0.0003              |
| 25 | 0.1229              | 0.0894              | 0.0892              | 0.0811              |
| 26 | -0.1465             | -0.1837             | -0.2373             | -0.2299             |
| 27 | 0.1448              | 0.1078              | 0.1006              | 0.1261              |
| 28 | -0.1554             | -0.1714             | -0.1603             | -0.1408             |
| 29 | -0.4798             | -0.2931             | -0.6835             | -0.2349             |
| 30 | 0.1448              | 0.1078              | 0.1006              | 0.1261              |
| 31 | -0.1554             | -0.1714             | -0.1603             | -0.1408             |
| 32 | 0.1448              | 0.1078              | 0.1006              | 0.1261              |
| 33 | -0.4798             | -0.2931             | -0.6835             | -0.2349             |
| 34 | 0.1448              | 0.1078              | 0.1006              | 0.1261              |
| 35 | 0.9712              | 0.8939              | 1.0299              | 0.7074              |
| 36 | 0.8384              | 0.6370              | 0.5175              | -0.4041             |
| 37 | -0.8384             | -0.6260             | -0.8898             | -0.8838             |
| 38 | -0.8340             | -0.8340             | -0.7762             | -0.8340             |
| 39 | 0.4170              | 0.4170              | 0.3665              | 0.4170              |
| 40 | 0.0600              | 0.0600              | 0.0600              | 0.0600              |
| 41 | -0.5700             | -0.5700             | -0.5400             | -0.5700             |
| 42 | 0.4200              | 0.4200              | 0.4600              | 0.4200              |
|   | 43   | 44   | 45   | 46   | 47   |
|---|------|------|------|------|------|
|   | 0.2950 | 0.2950 | 0.3850 | 0.2950 | 0.3850 |
|   | 0.2950 | 0.2950 | 0.3850 | 0.2950 | 0.3850 |
|   | 0.2950 | 0.2950 | 0.3850 | 0.2950 | 0.3850 |
|   | 0.1150 | 0.1150 | 0.1150 | 0.1150 | 0.1150 |
|   | 0.1150 | 0.1150 | 0.1150 | 0.1150 | 0.1150 |
|   | 0.1150 | 0.1150 | 0.1150 | 0.1150 | 0.1150 |
|   | -0.4900 | -0.4900 | -0.5400 | -0.4900 | -0.5400 |
|   | -0.4900 | -0.4900 | -0.5400 | -0.4900 | -0.5400 |
|   | -0.4900 | -0.4900 | -0.5400 | -0.4900 | -0.5400 |
|   | -0.0150 | -0.0150 | 0.2150 | -0.0150 | 0.2150 |
|   | -0.0150 | -0.0150 | 0.2150 | -0.0150 | 0.2150 |
|   | -0.0150 | -0.0150 | 0.2150 | -0.0150 | 0.2150 |
|   | 0.1150 | 0.1150 | 0.1150 | 0.1150 | 0.1150 |
|   | 0.1150 | 0.1150 | 0.1150 | 0.1150 | 0.1150 |
|   | 0.1150 | 0.1150 | 0.1150 | 0.1150 | 0.1150 |
Table S14: Bond Parameters for Covalent Bonds of the Reacting Part.

| Bond Type | $E_D$ (kcal·mol$^{-1}$) | $\alpha$ (Å$^2$) | $r_0$ (Å) | $k_b$ (kcal·mol$^{-1}$·Å$^{-2}$) | $b$ (Å) |
|-----------|------------------------|------------------|---------|---------------------------------|------|
| 0         | Not Set                |                  |         |                                 |      |
| 1         |                        | 734              | 1.0800  |                                 |      |
| 2         |                        | 938              | 1.4000  |                                 |      |
| 3         |                        | 634              | 1.5100  |                                 |      |
| 4         |                        | 680              | 1.0900  |                                 |      |
| 5         |                        | 536              | 1.5290  |                                 |      |
| 6         |                        | 640              | 1.4100  |                                 |      |
| 7         |                        | 634              | 1.5220  |                                 |      |
| 8         |                        | 428              | 1.3270  |                                 |      |
| 9         |                        | 1140             | 1.2290  |                                 |      |
| 10        |                        | 634              | 1.5040  |                                 |      |
| 11        |                        | 854              | 1.3810  |                                 |      |
| 12        |                        | 1040             | 1.3700  |                                 |      |
| 13        |                        | 868              | 1.0100  |                                 |      |
| 14        |                        | 954              | 1.3430  |                                 |      |
| 15        |                        | 976              | 1.3350  |                                 |      |
| 16        |                        | 820              | 1.3940  |                                 |      |
| 17        |                        | 1312             | 1.2500  |                                 |      |
| 18        |                        | 2000             | 0.9572  |                                 |      |
| 19        |                        | 900              | 1.3640  |                                 |      |
| 20        |                        | 1106             | 0.9450  |                                 |      |
| 21        |                        | 560              | 1.5100  |                                 |      |
| 22        |                        | 680              | 1.0880  |                                 |      |
| 23        |                        | 520              | 1.5090  |                                 |      |
| 24        |                        | 640              | 1.3800  |                                 |      |
| 25        | 80.00                  | 2.0              | 1.4100  |                                 |      |
| 26        | 80.00                  | 2.0              | 1.3800  |                                 |      |
| 27        | 284.72                 | 1.2              | 1.0100  |                                 |      |
| 28        | 245.78                 | 1.5              | 0.9572  |                                 |      |

Morse bonds (reacting atoms): $V_{\text{Morse}} = D_e \{1 - \exp[-\alpha (r_{ij} - r_0)]\}^2$; Harmonic bonds (non-reacting atoms): $V_{\text{Harmonic}} = 0.5k (r_{ij} - r_0)^2$. 

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Table S15: Bond Types in Different VB States.

| Number | Bond Type | #1  | #2  | C1 Attack | C2 Attack |
|--------|-----------|-----|-----|-----------|-----------|
|        |           | State I | State II | State III | State II | State III |
| 1      |           | 1    | 1    | 1         | 1         |
| 1      |           | 2    | 2    | 2         | 2         |
| 1      |           | 7    | 2    | 2         | 2         |
| 3      |           | 4    | 1    | 1         | 1         |
| 3      |           | 5    | 2    | 2         | 2         |
| 5      |           | 6    | 1    | 1         | 1         |
| 5      |           | 11   | 2    | 2         | 2         |
| 7      |           | 8    | 1    | 1         | 1         |
| 7      |           | 9    | 2    | 2         | 2         |
| 9      |           | 10   | 1    | 1         | 1         |
| 9      |           | 11   | 2    | 2         | 2         |
| 11     |           | 12   | 21   | 3         | 3         |
| 12     |           | 13   | 22   | 4         | 4         |
| 12     |           | 14   | 0    | 0         | 6         |
| 12     |           | 14   | 25   | 0         | 0         |
| 12     |           | 15   | 23   | 5         | 5         |
| 14     |           | 0    | 0    | 0         | 0         |
| 12     |           | 32   | 0    | 25        | 6         |
| 14     |           | 15   | 6    | 6         | 6         |
| 15     |           | 16   | 22   | 4         | 4         |
| 15     |           | 17   | 21   | 3         | 3         |
| 15     |           | 32   | 0    | 0         | 25        |
| 17     |           | 18   | 2    | 2         | 2         |
| 17     |           | 22   | 2    | 2         | 2         |
| 18     |           | 19   | 1    | 1         | 1         |
| 18     |           | 20   | 2    | 2         | 2         |
| 20     |           | 21   | 1    | 1         | 1         |
| 20     |           | 26   | 2    | 2         | 2         |
| 22     |           | 23   | 1    | 1         | 1         |
| 22     |           | 24   | 2    | 2         | 2         |
| 24     |           | 25   | 1    | 1         | 1         |
| 24     |           | 26   | 2    | 2         | 2         |
| 26     |           | 27   | 1    | 1         | 1         |
| 28     |           | 29   | 4    | 4         | 4         |
| 28     |           | 30   | 4    | 4         | 4         |
| 28     |           | 31   | 7    | 5         | 7         |
Table S16: Angle Parameters Used for Bending Adjacent Bonds in the Reacting Part.

| Angle Type | $k_a$ (kcal·mol⁻¹·rad⁻²) | $\Theta_0$ (°) | Angle Type | $k_a$ (kcal·mol⁻¹·rad⁻²) | $\Theta_0$ (°) |
|------------|--------------------------|----------------|------------|--------------------------|----------------|
| 0          | No Set                   |                |            |                          |                |
| 1          | 70.0                     | 120.00         | 17         | 140.0                    | 109.80         |
| 2          | 126.0                    | 120.00         | 18         | 140.0                    | 110.00         |
| 3          | 140.0                    | 120.00         | 19         | 140.0                    | 111.00         |
| 4          | 70.0                     | 109.50         | 20         | 70.0                     | 128.20         |
| 5          | 126.0                    | 114.00         | 21         | 140.0                    | 117.00         |
| 6          | 100.0                    | 109.50         | 22         | 140.0                    | 117.00         |
| 7          | 75.0                     | 110.70         | 23         | 160.0                    | 126.00         |
| 8          | 66.0                     | 107.80         | 24         | 400.0                    | 104.52         |
| 9          | 126.0                    | 111.10         | 25         | 70.0                     | 113.00         |
| 10         | 162.0                    | 111.40         | 26         | 75.0                     | 117.20         |
| 11         | 160.0                    | 120.40         | 27         | 75.0                     | 117.20         |
| 12         | 166.0                    | 123.40         | 28         | 60.0                     | 60.00          |
| 13         | 166.0                    | 116.90         | 29         | 185.2                    | 111.55         |
| 14         | 140.0                    | 121.60         | 30         | 120.0                    | 109.50         |
| 15         | 140.0                    | 130.70         | 31         | 70.0                     | 130.70         |
| 16         | 140.0                    | 106.30         | 32         | 110.0                    | 108.50         |

Angle potential: $V_{\text{angle}} = 0.5 \sum k (\Theta - \Theta_0)^2$
Table S17: Angle Types of the Different VB States.

| Atom Number | Angle Type | C1 Attack | C2 Attack |
|-------------|------------|-----------|-----------|
|             | #1 | #2 | #3 | State I | State II | State III | State II | State III |
| 1           | 1  | 3  | 4  | 1       | 1         | 1         | 1         | 1         |
| 1           | 1  | 3  | 5  | 2       | 2         | 2         | 2         | 2         |
| 1           | 1  | 7  | 8  | 1       | 1         | 1         | 1         | 1         |
| 1           | 1  | 7  | 9  | 2       | 2         | 2         | 2         | 2         |
| 2           | 2  | 1  | 3  | 1       | 1         | 1         | 1         | 1         |
| 2           | 2  | 1  | 7  | 1       | 1         | 1         | 1         | 1         |
| 3           | 3  | 1  | 7  | 2       | 2         | 2         | 2         | 2         |
| 3           | 3  | 5  | 6  | 1       | 1         | 1         | 1         | 1         |
| 3           | 3  | 5  | 11 | 2       | 2         | 2         | 2         | 2         |
| 4           | 4  | 3  | 5  | 1       | 1         | 1         | 1         | 1         |
| 5           | 5  | 11 | 9  | 2       | 2         | 2         | 2         | 2         |
| 5           | 5  | 11 | 12 | 3       | 3         | 3         | 3         | 3         |
| 6           | 6  | 5  | 11 | 1       | 1         | 1         | 1         | 1         |
| 7           | 7  | 9  | 10 | 1       | 1         | 1         | 1         | 1         |
| 7           | 7  | 9  | 11 | 2       | 2         | 2         | 2         | 2         |
| 8           | 8  | 7  | 9  | 1       | 1         | 1         | 1         | 1         |
| 9           | 9  | 11 | 12 | 3       | 3         | 3         | 3         | 3         |
| 10          | 10 | 9  | 11 | 1       | 1         | 1         | 1         | 1         |
| 11          | 11 | 12 | 13 | 4       | 4         | 4         | 4         | 4         |
| 11          | 11 | 12 | 14 | 6       | 0         | 0         | 6         | 6         |
| 11          | 11 | 12 | 15 | 5       | 5         | 5         | 5         | 5         |
| 11          | 11 | 12 | 32 | 0       | 6         | 6         | 0         | 0         |
| 12          | 12 | 14 | 15 | 28      | 0         | 0         | 0         | 0         |
| 12          | 12 | 15 | 14 | 28      | 6         | 6         | 0         | 0         |
| 12          | 12 | 15 | 16 | 0       | 0         | 0         | 7         | 7         |
| 12          | 12 | 15 | 16 | 27      | 7         | 7         | 0         | 0         |
| 12          | 12 | 15 | 17 | 5       | 5         | 5         | 5         | 5         |
| 12          | 12 | 15 | 32 | 0       | 0         | 0         | 6         | 6         |
| 13          | 13 | 12 | 14 | 0       | 0         | 0         | 4         | 4         |
| 13          | 13 | 12 | 31 | 0       | 13        | 30        | 0         | 0         |
| 13          | 13 | 12 | 14 | 26      | 0         | 0         | 0         | 0         |
| 13          | 13 | 12 | 15 | 27      | 7         | 7         | 0         | 0         |
| 13          | 13 | 12 | 32 | 0       | 4         | 4         | 0         | 0         |
| 14          | 14 | 12 | 15 | 28      | 0         | 0         | 0         | 0         |
| 14          | 14 | 15 | 16 | 26      | 4         | 4         | 0         | 0         |
| 14          | 14 | 15 | 17 | 6       | 6         | 6         | 0         | 0         |
| 15          | 15 | 12 | 32 | 0       | 6         | 6         | 0         | 0         |
| 13 | 12 | 15 | 0  | 0  | 0  | 7  | 7  |
|----|----|----|----|----|----|----|----|
| 14 | 12 | 15 | 0  | 0  | 0  | 6  | 6  |
| 14 | 15 | 16 | 0  | 0  | 0  | 0  | 0  |
| 14 | 15 | 17 | 0  | 0  | 0  | 0  | 0  |
| 15 | 17 | 18 | 3  | 3  | 3  | 3  | 3  |
| 15 | 17 | 22 | 3  | 3  | 3  | 3  | 3  |
| 15 | 32 | 31 | 0  | 0  | 0  | 0  | 13 | 30 |
| 16 | 15 | 17 | 4  | 4  | 4  | 4  | 4  |
| 16 | 15 | 32 | 0  | 0  | 0  | 4  | 4  |
| 17 | 15 | 32 | 0  | 0  | 0  | 0  | 6  |
| 17 | 18 | 19 | 1  | 1  | 1  | 1  | 1  |
| 17 | 18 | 20 | 2  | 2  | 2  | 2  | 2  |
| 17 | 22 | 23 | 1  | 1  | 1  | 1  | 1  |
| 17 | 22 | 24 | 1  | 1  | 1  | 1  | 1  |
| 18 | 17 | 22 | 1  | 1  | 1  | 1  | 1  |
| 18 | 20 | 21 | 1  | 1  | 1  | 1  | 1  |
| 18 | 20 | 26 | 2  | 2  | 2  | 2  | 2  |
| 19 | 18 | 20 | 1  | 1  | 1  | 1  | 1  |
| 20 | 26 | 24 | 1  | 1  | 1  | 1  | 1  |
| 20 | 26 | 27 | 1  | 1  | 1  | 1  | 1  |
| 21 | 20 | 26 | 1  | 1  | 1  | 1  | 1  |
| 22 | 24 | 25 | 1  | 1  | 1  | 1  | 1  |
| 22 | 24 | 26 | 1  | 1  | 1  | 1  | 1  |
| 23 | 24 | 24 | 1  | 1  | 1  | 1  | 1  |
| 24 | 26 | 27 | 1  | 1  | 1  | 1  | 1  |
| 25 | 24 | 26 | 1  | 1  | 1  | 1  | 1  |
| 28 | 31 | 32 | 22 | 10 | 6  | 10 | 6  |
| 28 | 31 | 33 | 22 | 11 | 6  | 11 | 6  |
| 28 | 31 | 34 | 0  | 0  | 0  | 0  | 6  |
| 29 | 28 | 30 | 8  | 8  | 8  | 8  | 8  |
| 29 | 28 | 31 | 4  | 4  | 7  | 4  | 7  |
| 30 | 28 | 31 | 4  | 4  | 7  | 4  | 7  |
| 31 | 34 | 36 | 0  | 0  | 0  | 0  | 32 |
| 32 | 31 | 33 | 23 | 12 | 29 | 12 | 29 |
| 32 | 31 | 34 | 0  | 0  | 0  | 29 | 0  |
| 33 | 31 | 34 | 0  | 0  | 29 | 0  | 29 |
| 35 | 34 | 36 | 24 | 24 | 0  | 24 | 0  |
| 37 | 40 | 41 | 14 | 14 | 14 | 14 | 14 |
| 37 | 40 | 46 | 15 | 15 | 15 | 15 | 15 |
| 38 | 37 | 39 | 8  | 8  | 8  | 8  | 8  |
| 38 | 37 | 40 | 4  | 4  | 4  | 4  | 4  |
| 39 | 37 | 40 | 4  | 4  | 4  | 4  | 4  |
| 40 | 41 | 42 | 1  | 1  | 1  | 1  | 1  |
Table 18: Torsion Parameters Used in the Reacting Part.

| Torsion Type | $V_1$  | $V_2$  | $V_3$  | Torsion Type | $V_1$  | $V_2$  | $V_1$  |
|--------------|--------|--------|--------|--------------|--------|--------|--------|
| 0            |        | 0.5    |        | 19           |        | 0.5    |        |
| 1            | 0.0000 | -3.6250| 0.0000 | 20           | 0.0000 | 2.3250 | 0.0000 |
| 2            | -1.0300| -0.1565| 0.1575 | 21           | 0.0000 | 5.0000 | 0.0000 |
| 3            | 0.8555 | -0.2500| 0.3315 | 22           | 0.0000 | 2.4000 | 0.0000 |
| 4            | 0.0000 | 0.0000 | 0.2310 | 23           | 1.5925 | -0.4125| 0.2465 |
| 5            | -0.8485| -0.2280| 0.2925 | 24           | 0.0000 | 0.0000 | -0.1125|
| 6            | 0.0000 | 0.0000 | 0.2340 | 25           | 0.0000 | 0.4100 | 0.0000 |
| 7            | 0.0000 | 0.0000 | 0.1500 | 26           | 0.3250 | -0.1250| 0.3350 |
| 8            | 2.1595 | 0.0000 | 0.0000 | 27           | 0.0000 | 0.0000 | 0.3800 |
| 9            | -0.6100| -0.0630| 0.2110 | 28           | 0.0000 | 0.0000 | -0.0500|
| 10           | 0.0000 | 0.0000 | 0.0990 | 29           | -0.6680| 0.0000 | 0.0000 |
| 11           | 0.0000 | 0.0000 | -0.2765| 30           | -0.2605| -1.0090| 0.9980 |
| 12           | -0.1385| 0.6140 | -0.3470| 31           | -0.1780| -0.0870| 0.2460 |
| 13           | 0.0000 | 0.0000 | 0.0660 | 32           | -0.6285| -0.9030| 0.0015 |
| 14           | 2.3345 | 2.5620 | 0.0000 | 33           | 0.0000 | 1.6000 | 0.0000 |
| 15           | 0.0000 | 2.5620 | 0.0000 | 34           | 0.5000 | 0.2730 | 0.2250 |
| 16           | 1.1830 | -0.1310| 0.2525 | 35           | 0.0000 | 0.2730 | 0.0000 |
| 17           | 0.0000 | 0.0000 | 0.2100 | 36           | 0.7500 | 2.7500 | 0.0000 |
| 18           | 0.0000 | 1.4000 | 0.0000 | 37           | 0.0000 | 2.7500 | 0.0000 |

$V_{\text{torsion}} = V_1 (1 + \cos(n\phi - \delta)) + V_2 (1 + \cos2(n\phi - \delta)) + V_3 (1 + \cos3(n\phi - \delta))$, $n$ is the periodicity (number of maxima per turn) and $\delta$ is the phase shift.$^{(a)}$
Table S19: Torsion Types in Different VB States.

| Atom Number | Torsion Type | C1 Attack | C2 Attack |
|-------------|--------------|-----------|-----------|
|             | State I      | State II  | State III | State II | State III |
| #1          | #2           | #3        | #4        |           |           |
| 1           | 7            | 9         | 10        | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 1           | 3            | 5         | 11        | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 1           | 7            | 9         | 11        | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 2           | 1            | 3         | 4         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 2           | 1            | 3         | 5         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 2           | 1            | 7         | 8         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 2           | 1            | 7         | 9         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 3           | 1            | 7         | 8         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 3           | 1            | 7         | 9         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 3           | 5            | 11        | 9         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 3           | 5            | 11        | 12        | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 4           | 3            | 5          | 6         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 4           | 3            | 5          | 11     | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 6           | 5            | 11        | 9         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 6           | 5            | 11        | 12        | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 7           | 1            | 3          | 4         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 7           | 1            | 3          | 5         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 7           | 9            | 11        | 5         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 7           | 9            | 11        | 12        | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 10           | 9            | 11        | 5         | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 10           | 11           | 12         | 1        | 1         | 1         | 1         | 1         | 1         | 1         | 1         | 1         |
| 11           | 12           | 15        | 14         | 26       | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
| 11           | 12           | 15        | 14         | 3         | 3         | 3         | 0         | 0         | 0         | 0         | 0         |
| 11           | 12           | 15        | 16         | 4         | 4         | 4         | 4         | 4         | 4         | 4         | 4         |
| 11           | 12           | 15        | 17         | 5         | 5         | 5         | 5         | 5         | 5         | 5         | 5         |
| 11           | 12           | 15        | 32         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
| 11           | 12           | 32        | 31         | 0         | 9         | 26       | 0         | 0         | 0         | 0         | 0         |
| 12           | 14           | 15        | 16         | 27       | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
| 12           | 14           | 15        | 17         | 26       | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
| 12           | 15           | 32        | 31         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
| 13           | 12           | 14        | 15         | 27       | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
| 13           | 12           | 15        | 14         | 6         | 6         | 6         | 6         | 6         | 6         | 6         | 6         |
| 13           | 12           | 15        | 16         | 7         | 7         | 7         | 7         | 7         | 7         | 7         | 7         |
| 13           | 12           | 15        | 17         | 4         | 4         | 4         | 4         | 4         | 4         | 4         | 4         |
|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| 13 | 12 | 32 | 31 | 0  | 10 | 27 |
| 14 | 12 | 15 | 16 | 6  | 0  | 0  |
| 14 | 12 | 15 | 17 | 3  | 0  | 0  |
| 14 | 12 | 15 | 32 | 0  | 0  | 8  |
| 15 | 12 | 32 | 31 | 0  | 9  | 26 |
| 15 | 17 | 18 | 19 | 1  | 1  | 1  |
| 15 | 17 | 18 | 20 | 1  | 1  | 1  |
| 15 | 17 | 22 | 23 | 1  | 1  | 1  |
| 15 | 17 | 22 | 24 | 1  | 1  | 1  |
| 16 | 15 | 32 | 31 | 0  | 0  | 10 |
| 17 | 15 | 32 | 31 | 0  | 0  | 9  |
| 17 | 18 | 20 | 21 | 1  | 1  | 1  |
| 17 | 18 | 20 | 26 | 1  | 1  | 1  |
| 17 | 18 | 20 | 25 | 1  | 1  | 1  |
| 18 | 17 | 22 | 23 | 1  | 1  | 1  |
| 18 | 17 | 22 | 24 | 1  | 1  | 1  |
| 18 | 20 | 26 | 24 | 1  | 1  | 1  |
| 18 | 20 | 26 | 27 | 1  | 1  | 1  |
| 19 | 18 | 20 | 21 | 1  | 1  | 1  |
| 19 | 18 | 20 | 26 | 1  | 1  | 1  |
| 21 | 20 | 26 | 24 | 1  | 1  | 1  |
| 21 | 20 | 26 | 27 | 1  | 1  | 1  |
| 22 | 17 | 18 | 19 | 1  | 1  | 1  |
| 22 | 17 | 18 | 20 | 1  | 1  | 1  |
| 22 | 24 | 26 | 20 | 1  | 1  | 1  |
| 22 | 24 | 26 | 27 | 1  | 1  | 1  |
| 23 | 22 | 24 | 25 | 1  | 1  | 1  |
| 23 | 22 | 24 | 26 | 1  | 1  | 1  |
| 25 | 24 | 26 | 20 | 1  | 1  | 1  |
| 25 | 24 | 26 | 27 | 1  | 1  | 1  |
| 28 | 31 | 32 | 12 | 0  | 14 | 26 |
| 28 | 31 | 32 | 15 | 0  | 0  | 14 |
| 28 | 31 | 34 | 36 | 0  | 0  | 31 |
| 29 | 28 | 31 | 32 | 0  | 13 | 6  |
| 29 | 28 | 31 | 33 | 0  | 0  | 6  |
| 29 | 28 | 31 | 34 | 0  | 0  | 6  |
| 30 | 28 | 31 | 32 | 0  | 13 | 6  |
| 30 | 28 | 31 | 33 | 0  | 0  | 6  |
| 30 | 28 | 31 | 34 | 0  | 0  | 6  |
| 32 | 12 | 15 | 14 | 0  | 8  | 8  |
| 32 | 12 | 15 | 16 | 0  | 6  | 6  |
| 32 | 12 | 15 | 17 | 0  | 3  | 3  |
|   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
| 32| 31| 34| 36|   |   | 32| 32|
| 33| 31| 32| 12|   |   | 30| 32|
| 33| 31| 32| 15|   |   | 33| 30|
| 33| 31| 34| 36|   |   | 32| 32|
| 34| 31| 32| 12|   |   | 30| 32|
| 34| 31| 32| 15|   |   | 33| 30|
| 35| 45| 46| 47|   |   | 33| 33|
| 37| 40| 41| 42| 18|   | 37| 37|
| 37| 40| 41| 43| 18|   | 37| 40|
| 38| 37| 40| 41| 17|   | 38| 37|
| 39| 37| 40| 41| 17|   | 39| 40|
| 40| 41| 43| 44| 20|   | 40| 41|
| 41| 40| 41| 43| 19|   | 41| 41|
| 41| 40| 46| 47| 19|   | 41| 41|
| 41| 46| 47| 19|   |   | 41| 41|
| 41| 43| 45| 46| 21|   | 41| 43|
| 41| 43| 45| 35|   |   | 41| 43|
| 42| 41| 43| 44| 20|   | 42| 41|
| 42| 41| 43| 45| 20|   | 42| 42|
| 43| 45| 46| 40| 22|   | 43| 45|
| 43| 45| 46| 47| 22|   | 43| 45|
| 44| 43| 45| 46| 21|   | 44| 43|
| 44| 43| 45| 35|   |   | 44| 43|
| 46| 40| 41| 42| 18|   | 46| 40|
| 46| 40| 41| 43| 18|   | 46| 46|
| R1| 28| 31| 32| 25|   | R1| 28|
| R1| 28| 31| 33| 25|   | R1| 28|
| R2| 37| 40| 41| 16|   | R2| 37|

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Table S20: Improper Torsion Parameters.

| Improper Type | $k_a$ (kcal·mol$^{-1}$·rad$^{-2}$) | $\tau_0$ (°) |
|---------------|---------------------------------|-------------|
| 0             | Not Set                         |             |
| 1             | 1.1                             | 180         |
| 2             | 10.5                            | 180         |
| 3             | 1.0                             | 180         |

Improper torsion potential: $V_{\text{torsion}} = k (\tau - \tau_0)^2$. $k$ is the force constant and $\tau$ is the equilibrium angle (in degrees).

Table S21: Improper Torsion Angles of the Different VB States.

| #1 | #2 | #3 | #4 | State I | State II | State III |
|----|----|----|----|---------|----------|-----------|
| 1  | 3  | 5  | 4  | 1       | 1        | 1         |
| 1  | 7  | 9  | 8  | 1       | 1        | 1         |
| 3  | 1  | 7  | 2  | 1       | 1        | 1         |
| 11 | 5  | 3  | 6  | 1       | 1        | 1         |
| 11 | 9  | 7  | 10 | 1       | 1        | 1         |
| 12 | 11 | 5  | 9  | 1       | 1        | 1         |
| 15 | 17 | 18 | 22 | 1       | 1        | 1         |
| 17 | 18 | 19 | 1  | 1       | 1        | 1         |
| 17 | 22 | 24 | 23 | 1       | 1        | 1         |
| 18 | 20 | 26 | 21 | 1       | 1        | 1         |
| 20 | 26 | 24 | 27 | 1       | 1        | 1         |
| 22 | 24 | 26 | 25 | 1       | 1        | 1         |
| 28 | 31 | 32 | 33 | 2       | 2        | 0         |
| 37 | 40 | 41 | 41 | 1       | 1        | 1         |
| 40 | 41 | 43 | 42 | 3       | 3        | 3         |
| 40 | 46 | 47 | 45 | 1       | 1        | 1         |
| 43 | 45 | 46 | 35 | 0       | 0        | 3         |
| 44 | 43 | 41 | 45 | 1       | 1        | 1         |
S5. Cartesian Coordinates for Key Stationary Points

Optimized stationary points along the reaction profiles of the nucleophilic attack of acetate on (S,S)-TSO. RS, TS, and PS denote reactant, transition and product states respectively.

Reactant

| Element | X         | Y         | Z         |
|---------|-----------|-----------|-----------|
| C       | -4.54071000 | -2.80493400 | 0.25243300 |
| H       | -5.10939500 | -3.69277100 | 0.00037200 |
| C       | -3.49485400 | -2.88013900 | 1.17225800 |
| H       | -3.24790400 | -3.82676200 | 1.63948000 |
| C       | -2.76840600 | -1.73599500 | 1.49991700 |
| H       | -1.96130000 | -1.79738400 | 2.22260000 |
| C       | -3.07323600 | -0.50654800 | 0.90441600 |
| C       | -4.12788700 | -0.43630900 | -0.01375900 |
| H       | -4.38283000 | 0.51597800  | -0.46436100 |
| C       | -4.85652500 | -1.57919900 | -0.33649900 |
| H       | -5.67389000 | -1.51295100 | -1.04583500 |
| C       | -2.26185300 | 0.69230800  | 1.25349200  |
| H       | -1.70870100 | 0.62069800  | 2.18768300  |
| C       | -1.70286500 | 1.61567500  | 0.23176600  |
| H       | -1.94776900 | 1.38878800  | -0.80381700 |
| O       | -2.84320500 | 1.98872000  | 1.02561300  |
| C       | -0.43604000 | 2.37256300  | 0.43154600  |
| C       | 0.53491400  | 2.35925600  | -0.57650000 |
| H       | 0.34527300  | 1.81852800  | -1.49795100 |
| C       | -0.18730600 | 3.08808700  | 1.60899700  |
| H       | -0.94245600 | 3.11900400  | 2.38593000  |
| C       | 1.74161200  | 3.03644900  | -0.40516700 |
| H       | 2.48630300  | 3.01722200  | -1.19281900 |
| C       | 1.01672500  | 3.76893800  | 1.77689400  |
| H       | 1.19782300  | 4.32319100  | 2.69103400  |
| C       | 1.98605000  | 3.74272600  | 0.77258000  |
| H       | 2.92183200  | 4.27369700  | 0.90509200  |
| C       | 7.41140900  | -3.41615600 | -2.64784400 |
| H       | 7.50651100  | -3.54972200 | -3.72634900 |
| H       | 8.13225900  | -2.65754300 | -2.32590100 |
| H       | 7.67428200  | -4.34734500 | -2.13965000 |
| C       | 5.99475100  | -2.96668700 | -2.24670900 |
| O       | 5.21566000  | -2.59766700 | -3.16794300 |
| O       | 5.72621700  | -2.98675900 | -1.01307800 |
TS

C   -3.36325800  -1.51569100  -1.81448300
H   -4.00426800  -2.36582800  -2.01913500
C   -2.66945800  -1.43368600  -0.60701300
H   -2.76920700  -2.21985600  0.13305200
C   -1.85066200  -0.33705100  -0.34781600
H   -1.31460000  -0.27539200  0.59321500
C   -1.70468600  0.69335500  -2.75708900
H   -3.77005800  -0.54892200  -3.69604800
C   -0.81354900  1.82251600  -0.96952600
H   -0.46890800  1.87789800  0.05198100
C   -0.89443500  3.13961600  -1.64379600
H   -1.07302400  3.05990200  -2.72508000
O   -2.03338700  3.39471600  -0.88695000
C   0.27462700  4.08699600  -1.42174700
C   1.07105300  4.50316800  -2.49266300
H   0.85516500  4.13355900  -3.48967400
C   0.56000200  4.58296300  -0.14429500
H   -0.06783300  4.28815800  0.68919700
C   2.13767500  5.38216000  -2.29463300
H   2.74316400  5.69612200  -3.13828600
C   1.62597800  5.45785800  0.06043400
H   1.83505400  5.83074000  1.05759900
C   2.42101700  5.86002400  -1.01536200
H   3.24788300  6.54387900  -0.85796500
C   2.86045700  0.04576100  -2.47285000
H   3.09231300  -0.36935900  -3.45428400
H   3.60900200  0.80712700  -2.23209400
C   2.92888000  -0.73498900  -1.71303600
C   1.47303400  0.69665200  -2.47040600
O   0.93099900  0.98677700  -3.55008000
O   0.99328700  0.90762700  -1.29794900

Product

C   -3.19453  -3.50771  -0.18685
H   -3.90255  -4.30983  -0.36372
C   -2.55518  -3.39370  1.04673
H   -2.76487  -4.10532  1.83783
C   -1.64929  -2.35551  1.26721
H   -1.16285  -2.26685  2.23365
C   -1.35997  -1.41997  0.26610
C   -2.01047  -1.54557  -0.96726
|   | X       | Y       | Z       |
|---|---------|---------|---------|
| H | -1.80184| -0.83512| -1.75586|
| C | -2.91996| -2.57774| -1.19142|
| H | -3.41631| -2.65676| -2.15267|
| C | -0.34843| -0.32699|  0.56175|
| H | -0.33770| -0.16625|  1.64000|
| C | -0.63690|  1.06571| -0.07696|
| H | -0.68190|  0.92193| -1.17367|
| O | -1.80851|  1.54759|  0.45065|
| C |  0.55112|  2.01601|  0.15590|
| C |  1.21028|  2.61360| -0.92336|
| H |  0.90727|  2.35777| -1.93466|
| C |  0.95917|  2.37072|  1.45016|
| H |  0.45586|  1.93456|  2.30669|
| C |  2.24376|  3.53442| -0.72627|
| H |  2.73516|  3.98737| -1.58131|
| C |  1.99313|  3.28148|  1.65651|
| H |  2.29550|  3.53580|  2.66736|
| C |  2.64161|  3.87039|  0.56645|
| H |  3.44398|  4.58240|  0.72637|
| C |  2.91520| -1.74337| -0.72360|
| H |  3.39315| -1.75673| -1.70159|
| H |  3.51688| -1.17149| -0.01611|
| H |  2.84485| -2.76822| -0.34778|
| C |  1.52417| -1.16611| -0.84064|
| O |  0.93027| -1.02851| -1.89145|
| O |  1.03640| -0.85290|  0.36368|

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