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Computational Studies on the Reactivity, Selectivity and Molecular Dynamics of Cycloaddition Reactions

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Chemistry

by

Peiyuan Yu

2017
ABSTRACT OF THE DISSERTATION

Computational Studies on the Reactivity, Selectivity and Molecular Dynamics of
Cycloaddition Reactions

by

Peiyuan Yu
Doctor of Philosophy in Chemistry
University of California, Los Angeles, 2017
Professor Kendall N. Houk, Chair

The first part of this dissertation describes computational studies of higher-order
cycloadditions with a focus on elucidating the origins of periselectivity. These reactions involve
ambimodal transition states (TS) and bifurcations of potential energy surface.

Chapter 1 describes density functional theory (DFT) studies of a transannular [6+4]
cycloaddition proposed in the biosynthesis of heronamide A, in which an unbridged 10-
membered ring is formed. Ring and steric strains are found to be essential in controlling the
product stability that makes this reaction feasible. Chapter 2 presents an Environment-Perturbed
Transition State Sampling method to explore the mechanism of the enzyme SpnF-catalyzed
Diels–Alder. A [6+4] cycloaddition is also involved and enzyme enhances the formation of
[4+2] product, with respect to the counterparts in the gas phase and in water. Chapter 3 explores
the mechanisms and selectivities of the cycloadditions of tropone to dimethylfulvene discovered
in 1967 by Houk. The two proposed pathways by which a key intermediate are formed are united through the discovery of an ambimodal $[6+4]/[4+6]$ TS using DFT calculations and molecular dynamics (MD) simulations.

The second part of this dissertation describes computational studies of Diels–Alder reactions and 1,3-dipolar cycloadditions using DFT calculations, with a focus on the mechanisms and the origins of regio- and stereoselectivities.

Chapter 4 reports the biochemical characterization of a Diels–Alderase found in the biosynthetic pathway of the cytotoxic myceliothermophin natural products. A theozyme model rationalizes both the substrate- and stereoselectivity of the enzyme. Chapter 5 discusses the concerted versus stepwise mechanisms of a series of dehydro-Diels–Alder (DDA) reactions. The reactivity of DDA reactions is controlled by the distortion energies required to achieve the TS geometries. Chapter 6 explores the mechanisms of Lewis acid-catalyzed Diels–Alder reactions of aryl allenes and acrylates. A stepwise mechanism involving short-lived zwitterion intermediates is established. The $[2+2]$ cycloaddition is not observed experimentally because of the greater charge separation in the first step of the $[2+2]$ cycloaddition. Chapter 7 focuses on the regioselectivity of 1,3-dipolar cycloadditions of benzo and mesitonitrile oxides with alkynyl pinacol and MIDA boronates. Calculated relative free energies of activation reproduce the experimentally observed product ratios. The electronic energies of activation are mainly controlled by distortion energies. Chapter 8 describes DFT studies of the first example of diazo esters as dienophiles in intramolecular Diels–Alder reactions with dienes. For comparison, the reactivities of diazo esters as 1,3-dipoles with dienes have also been explored. The usually observed 1,3-dipolar cycloaddition was not observed because of strong tether distortion in the $(3+2)$ TS.
The dissertation of Peiyuan Yu is approved.

Neil Kamal Garg

Jennifer M. Murphy

Kendall N. Houk, Committee Chair

University of California, Los Angeles

2017
DEDICATION

To my parents, to whom I owe most, for their unconditional love and support...
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I am extremely grateful to my parents, to whom this dissertation is dedicated. I want to thank my girlfriend, Yi, who is always supportive and inspiring to me, to become a better person, in work and in life.

Chapter 2 describes the study of SpnF-catalyzed Diels–Alder reaction in condensed media. Zhongyue Yang developed the EPTSS method and performed reaction dynamics simulations, Song Yang performed protein dynamics simulations, I performed QM/MM optimizations and energy calculations in water, and Dr. Yanwei Li performed optimizations and energy calculations in the enzyme, while Professor Houk supervised the research.


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CHEM 30C (Organic Chemistry III: Reactivity, Synthesis, and Biomolecules)

Publications


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Presentations

1. Yu, P.; Li, W.; Houk, K. N. Theoretical Study of a Novel 1,5-Acyl Shift of Allylic Esters. Seaborg Symposium, UCLA, Los Angeles, 2016. (Poster presentation)


4. Yu, P.; Zhong, G. Development of New Organocatalytic Reactions. DISCOVER URECA @ NTU Poster Exhibition and Competition, 2010. (Poster presentation)
Chapter 1. Transannular [6+4] and Ambimodal Cycloaddition in the Biosynthesis of Heronamide A

1.1 Abstract

The transannular [6+4] cycloaddition proposed as a step in the biosynthesis of heronamide A has been modeled using density functional theory. The proposed cycloaddition is highly stereoselective, affording a single product. The reaction proceeds through an ambimodal transition state that directly leads to a [4+2] adduct in addition to the observed [6+4] adduct. Interconversion of these adducts is possible via a facile Cope rearrangement. The [6+4] adduct is thermodynamically more stable than the [4+2] adduct by 5.2 kcal mol$^{-1}$ due to a combination of the ring and steric strain in the [4+2] product. The results strongly support the plausibility of the proposed transannular [6+4] cycloaddition in the biogenesis of heronamide A and may provide insights to designing substrates that selectively undergo [6+4] cycloaddition to form unbridged 10-membered rings.

1.2 Introduction

Woodward and Hoffmann first predicted that thermal [6+4] cycloadditions were allowed pericyclic transformations in 1965, when no cycloadditions involving 10 electrons were known.\textsuperscript{1,2} In 1966, the first observations of a [6+4] cycloadduct occurred independently in the laboratories of Cookson\textsuperscript{3} and Ito.\textsuperscript{4} Now it seems that nature enlisted this reaction in biosynthesis long before chemists discovered it. In the nearly 5 decades since the Cookson–Ito reports, the mechanism and synthetic utility of this reaction have been studied widely.\textsuperscript{5-13} Despite these studies, the utility of [6+4] cycloaddition has remained rather limited, especially in comparison
to the ubiquitous Diels–Alder reaction. The 6π-electron cycloaddends utilized in [6+4] cycloadditions have generally been limited to cyclic trienes such as cycloheptatrienes, fulvenes, and tropones. As a result, the cycloadducts resulting from these reactions all contain bridged 10-membered ring structures, and the formation of unbridged 10-membered rings by [6+4] cycloadditions remains unrealized. Alder et al. have modeled computationally a number of [6+4] cycloadditions to explain the origins of this limitation and to propose substrates that may undergo the desired cycloaddition.\(^{14}\) They find that often a competing Diels–Alder cycloaddition is more facile, and in many of the cases where the [6+4] adduct is predicted to form, it can rapidly rearrange via a Cope rearrangement to yield the more stable [4+2] adduct. The biosynthesis of heronamide A suggests that nature has long solved the synthetic challenge of constructing unbridged 10-membered rings by [6+4] cycloaddition.

In 2010, Capon et al. isolated a new 20-membered polyene macrolactam, heronamide C (1), from fermentations of a marine-derived Streptomyces sp. (CMB-M0406), along with heronamide A (2) and heronamide B, both putatively derived from 1.\(^{15}\) After initial misassignment of the structures of these heronamides, spectroscopic\(^{16}\) and synthetic\(^{17}\) studies determined that the heronamides feature conserved stereochemistry, in support of the notions these species may be formed via a common biosynthetic pathway. Sugiyama and co-workers reported further proof of this when they demonstrated that 1 could spontaneously convert into 2 in DMSO.\(^{18}\) Recently, Zhang et al. reported that heronamides D–F, which possess a two-carbon-shorter side chain compared with that of heronamides A–C, exhibit similar biosynthetic relationship.\(^{19}\)

As shown in Scheme 1.1, the biosynthetic pathway proposed by Capon involves the site-selective oxidation of 1 to form epoxide 3. Polyenes are known to be air-oxidized to afford
epoxide derivatives spontaneously\textsuperscript{20} or enzymatically.\textsuperscript{21} The subsequent epoxide ring opening of 3 via S\textsubscript{N}2 attack of the amide nitrogen to form 4 is also likely to be feasible because of proximity and strain release.\textsuperscript{22} The last step of the proposed pathway is an unprecedented transformation in which an unbridged 10-membered ring is constructed via a transannular [6+4] cycloaddition. We have explored this step using DFT computations.

**Scheme 1.1.** Revised structures of heronamide C (1) and heronamide A (2) and the proposed biosynthetic relationship between them

![Scheme 1.1](image)

**1.3 Computational Methods**

All density functional theory computations were performed using Gaussian09.\textsuperscript{23} Geometry optimizations and subsequent frequency calculations were performed at the B3LYP/6-
31G(d) level of theory,\textsuperscript{24} both with and without Grimme’s D3 empirical dispersion correction.\textsuperscript{25} The empirical dispersion correction was found to have little effect on both the geometries and energies of stationary points; therefore we have elected to report structures optimized at the B3LYP/6-31G(d) model chemistry. Normal vibrational mode analysis confirmed optimized structures were minima or transition states. Zero point vibrational energy (ZPE) and thermal corrections were calculated using unscaled B3LYP/6-31G(d) frequencies. Truhlar’s quasiharmonic correction was used to compute molecular entropies to reduce error caused by the breakdown of the harmonic approximation, by setting all positive frequencies that are less than 100 cm\textsuperscript{–1} to 100 cm\textsuperscript{–1}.\textsuperscript{26} Since the M06-2X method\textsuperscript{27} has been shown to yield more accurate energetics for cycloaddition reactions,\textsuperscript{28} M06-2X/6-311+G(d,p) single point energies were computed on the B3LYP-optimized structures. For the model reaction, solvent effects were evaluated with the CPCM polarizable conductor model.\textsuperscript{29} Reported energies are Gibbs free energies determined by summing these single point electronic energies and ZPE and thermal corrections determined using B3LYP/6-31G(d). All 3D renderings of stationary points were generated using CYLview.\textsuperscript{30} Gaussview\textsuperscript{31} and Avogadro\textsuperscript{32} were used to construct the structures used in our computations.

Conformational searches of the ground state structures for all reactants, intermediates and products were performed using the MMFF force field. A low mode/Monte Carlo (LMMC) search protocol optimized for sampling of macrocycles available in Macromodel 9.9 was performed.\textsuperscript{33} Up to fifty lowest-energy structures in each case were then used as starting points for DFT optimization using B3LYP/6-31G(d) level of theory.

\textbf{1.4 Results and Discussion}
1.4.1 Model reaction, transition structure, and free energy diagram

To reduce the time required for individual computation and to reduce the number of conformers that need to be sampled, we modeled the [6+4] cycloaddition of a truncated substrate, in which the octa-2,4-dien-1-yl side chain of heronamide A (and related compounds) was replaced with a methyl group.

Scheme 1.2 shows the [6+4] cycloaddition of model substrate 5. The reaction is computed to be exergonic by 16.9 kcal mol\(^{-1}\). To investigate the stereoselectivity of the reaction, 12 different transition states were located with the lowest energy one shown in Figure 1.1 (TS-1, \(\Delta G^\ddagger = 20.8\) kcal mol\(^{-1}\)). The energies of the transition states leading to alternative products range from 23.3 to 40.4 kcal mol\(^{-1}\). The energy difference between TS-1 and the second lowest energy transition state is 2.5 kcal mol\(^{-1}\). This is in agreement with the experimental finding that 2 is formed exclusively. We also located a second transition state involving formation of only one bond, leading to a diradical intermediate. However, it is about 10 kcal mol\(^{-1}\) higher in energy than TS-1. The activation free energy for this nonenzymatic reaction is 20.8 kcal mol\(^{-1}\). At 298 K, the reaction has a computed half-life (\(t_\text{1/2}\)) of about 3 minutes. In comparison, the experimentally observed transformation of 1 to 2 takes days.\(^{18}\) Therefore, it is unlikely that intermediate 4 could be observed prior to cycloaddition, and an earlier biosynthetic step is probably rate limiting.

Scheme 1.2. Model reaction scheme and computed reaction free energy
Figure 1.1. B3LYP/6-31G(d)-optimized transition structure for the ambimodal cycloaddition of 5. Energies reported are M06-2X/6-311+G(d,p)//B3LYP/6-31G(d) free energies in kcal mol$^{-1}$.

As shown in Figure 1.1, **TS-1** is highly asynchronous; the shortest partially formed sigma bond having a forming bond length of 1.95 Å, and the second partially formed sigma bond having a forming bond length of 2.91 Å. **TS-1** has a third partially formed sigma bond with a forming bond length of 3.16 Å, which corresponds to one of the two bonds that are formed by a [4+2] cycloaddition of 5. Although we made many attempts, we were not able to locate separate TS structures for this [4+2] cycloaddition. The [6+4] and [4+2] pathways are merged at **TS-1**. Such a transition state is described as “ambimodal”, as it leads directly to multiple products.$^{34}$ The [6+4]/[4+2] ambimodal transition state is bis-pericyclic in nature, with two sets of stabilizing cyclic aromatic orbital interactions.$^{35}$

Figure 1.2a shows the free energy diagram of the ambimodal cycloaddition of 5. A valley-ridge inflection (VRI) point might exist along this pathway, where the potential energy surface (PES) bifurcates, leading to two distinct adducts: the observed [6+4] adduct 6 and a [4+2] adduct 7. The exact definition of a VRI point has been debated in the literature.$^{36}$ A more general concept, the so-called “valley-ridge transition (VRT) point”, has been proposed recently.$^{37}$ The VRT point can be located unequivocally along the intrinsic reaction coordinate.
(IRC) of reactions that involve bifurcations. Figure 1.2b shows the plot of energies of points along the forward IRC. To locate approximately the VRT point, we took every point along the IRC and computed the projected frequencies for vibrations perpendicular to the path. After the point indicated as “VRT” in Figure 1.2b, one imaginary frequency is found at each point. In other words, the valley becomes a ridge after this point. The product distribution of reactions occurring of bifurcating surfaces cannot be determined solely by transition state theory. Instead, the shape of the PES and resulting dynamical effects will play an important role. For the reaction of 5, the [4+2] adduct 7 is calculated to be 5.2 kcal mol\(^{-1}\) less stable than the [6+4] adduct 6. A possible Cope rearrangement reaction could convert 7 into 6. Indeed, we located the Cope rearrangement transition state TS-2. It has a barrier of 24.5 kcal mol\(^{-1}\). Therefore, starting from substrate 5, the [4+2] intermediate 7 should be observable, but will convert to the more stable [6+4] adduct at room temperature. The IRC leads to a conformer of 6 (shown in Figure 1.2b, the last point on the IRC, where the cyclohexene ring adopts a boat conformation). This is in agreement with the behavior of most unsymmetrical bifurcating potential energy surfaces, where the calculated minimum energy path (MEP) leads to only one product. In contrast, for symmetrical bifurcating PES such as the endo dimerization of cyclopentadiene, the MEP leads to the second TS. To provide further evidence for the connection between TS-1 and the unobserved [4+2] product 7 without undertaking dynamic trajectory studies for such a large system, we designed a truncated model substrate 5t, for which the [6+4] and [4+2] products are same in energy, and the PES after the VRT point is symmetrical. Indeed, the IRC that follows the ambimodal cycloaddition TS does lead to the Cope TS: a hallmark of symmetrical bifurcating PES.
Figure 1.2. (a) Free energy diagram of the cycloaddition of macrolactam 5 featuring an ambimodal TS-1 that leads to the [6+4] and [4+2] adducts 6 and 7, respectively. 7 can convert to 6 through Cope rearrangement via TS-2. Energies reported are M06-2X/6-311+G(d,p)//B3LYP/6-31G(d) free energies in kcal mol\(^{-1}\). Solvation has only a minor effect on the barriers of reaction and does not affect the relative stability of the two cycloadducts. Energies in parentheses are CPCM(Water)-M06-2X/6-311+G(d,p)//B3LYP/6-31G(d) free energies in kcal mol\(^{-1}\).
mol$^{-1}$. (b) Plot of energies (B3LYP/6-31G(d)) versus points along the computed forward IRC starts from TS-1.

Unlike the transannular cycloaddition of 5, for which the [6+4] adduct 6 is more stable than the [4+2] adduct 7, the ambimodal cycloaddition of 1,3,5-hexatriene and 1,3-butadiene yields the [4+2] adduct when under thermodynamic control.$^{14}$ To understand influence of substitution of the cycloaddends on the equilibrium between 6 and 7, we modeled the cycloaddition of a series of model substrates. The results are summarized below.

### 1.4.2 Effects of tethers on the barrier of reaction and relative stability of the [6+4] and [4+2] cycloadducts

Table 1.1 shows the computed reaction barriers and relative stability of adducts formed by the ambimodal cycloadditions of different substrates. These model substrates were chosen to interrogate the role of tethers and substituents on the reaction barriers and relative stability of the cycloadducts. For the cycloaddition of unsubstituted butadiene 8 and hexatriene 9, the [6+4] adduct 10 is less stable than the [4+2] adduct 11 by 6.4 kcal mol$^{-1}$ due to the strain of the medium-sized ring. The reaction barrier decreases by 4.3 kcal mol$^{-1}$ when the reaction changes from intermolecular to intramolecular. The three-carbon tether connected to C-9 and C-10 (13, shown in red) has little effect on the instability of the ten-membered ring relative to the six-membered isomer. However, when a four carbon tether is added at the C-5 and C-6 positions of the ten-membered ring, the relative stability of the two adducts is reversed. The [6+4] adduct 16 is now more stable than the [4+2] adduct 17. Because of the four-carbon tether, both adducts contain strained ten-membered rings. The small preference (1.2 kcal mol$^{-1}$) for 16 is likely due to
the alkene conjugation, which is not present in 17. An additional three-membered tether at the C-9 and C-10 positions has no effect on the relative stability of 19 and 20 (1.2 kcal mol$^{-1}$). An additional double bond in the four-carbon tether slightly stabilizes the [4+2] adduct (the difference becomes to 0.3 kcal mol$^{-1}$), probably due to additional alkene conjugation in 23 compared to 20. When the full amide tether is added (24), the reaction barrier drops significantly by 6.1 kcal mol$^{-1}$. This is not totally unexpected, because of the electron-withdrawing nature of the carbonyl group. The same small preference for the [6+4] product is preserved (1.4 kcal mol$^{-1}$) when the full tethers are added (27). Thus, the four-carbon tether linking C-5 and C-6 destabilizes the [4+2] adduct by introducing a strained medium-sized ring in this product; while the amide tether is responsible for lowering the barrier of this reaction. Based on these results, the tether effects are only partially responsible for the large energy difference between 6 and 7 (5.2 kcal mol$^{-1}$): a 10:1 mixture of these adducts would be expected at room temperature (based on the energy difference between 28 and 29).
Table 1.1. Effects of tethers and substituents on the reaction barriers and the relative stability of the [6+4] and [4+2] cycloadducts.\textsuperscript{a}

<table>
<thead>
<tr>
<th>substrate(s)</th>
<th>TS\textsubscript{[6+4]/[4+2]}</th>
<th>[6+4] adduct</th>
<th>[4+2] adduct</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 9</td>
<td>TS-3</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>0.0</td>
<td>32.7</td>
<td>-12.2</td>
<td>-18.7 (-6.4)</td>
</tr>
<tr>
<td>12</td>
<td>TS-4</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>0.0</td>
<td>28.4</td>
<td>-14.0</td>
<td>-20.0 (-6.0)</td>
</tr>
<tr>
<td>15</td>
<td>TS-5</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>0.0</td>
<td>26.6</td>
<td>-19.3</td>
<td>-18.1 (1.2)</td>
</tr>
<tr>
<td>18</td>
<td>TS-6</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>0.0</td>
<td>24.8</td>
<td>-18.2</td>
<td>-17.0 (1.2)</td>
</tr>
<tr>
<td>21</td>
<td>TS-7</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>0.0</td>
<td>25.9</td>
<td>-11.2</td>
<td>-10.9 (0.3)</td>
</tr>
<tr>
<td>24</td>
<td>TS-8</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>0.0</td>
<td>19.8</td>
<td>-16.3</td>
<td>-15.5 (0.8)</td>
</tr>
<tr>
<td>27</td>
<td>TS-9</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>0.0</td>
<td>20.7</td>
<td>-16.9</td>
<td>-15.4 (1.4)</td>
</tr>
<tr>
<td>30</td>
<td>TS-10</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>0.0</td>
<td>19.9</td>
<td>-15.2</td>
<td>-13.7 (1.5)</td>
</tr>
<tr>
<td>33</td>
<td>TS-11</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>0.0</td>
<td>21.3</td>
<td>-17.9</td>
<td>-16.2 (1.7)</td>
</tr>
</tbody>
</table>
1.4.3 Effects of methyl substituents on the relative stability of the [6+4] and [4+2] cycloadducts

We next probed the effects of methyl substituents on 6 and 7. As shown in Figure 1.3, all methyl groups on the ten-membered rings adopt pseudoaxial orientations. A single methyl group at the C-4 or C-8 position has little effect on the equilibrium between the [6+4] and [4+2] adducts (Table 1.1). The energy differences between 31 and 32 (1.5 kcal mol\(^{-1}\)) as well as 34 and 35 (1.7 kcal mol\(^{-1}\)) are virtually identical to that of 28 and 29 (1.4 kcal mol\(^{-1}\)). For 31, the stabilizing effect of a methyl group on the double bond is likely cancelled by increased allylic 1,3-strain\(^{41}\) in 31 as compared to 32 (Figure 1.3, 1.97 Å vs. 2.09 Å). However, when both methyl substituents are present on the cycloaddends, as in 5, the resulting [4+2] adduct 7 is now less stable than the [6+4] adduct 6 by 5.2 kcal mol\(^{-1}\). The hydrogen atom distance indicated in 7 is 2.19 Å, where as the two methyl groups are separated far away from each other in 6. The 3.5~3.8 kcal mol\(^{-1}\) increase in relative energies compared with analogues of monomethyl or lacking methyl is the result of steric interactions between the two methyl groups in 7. For comparison, the 1,3-diaxial methyl-methyl interactions measured in a substituted chair cyclohexane are destabilizing by 3.7 kcal mol\(^{-1}\),\(^{42}\) and the methyl-methyl repulsion is similar in 7.
1.5 Conclusion

The unprecedented transannular [6+4] cycloaddition in a proposed biosynthetic pathway of heronamide A has been investigated by density functional theory (DFT) computations. Calculations indicate that the [6+4] cycloaddition is stereoselective and proceeds through an ambimodal transition state that can lead to both the observed [6+4] and an unobserved [4+2] product. However, the [4+2] product is less stable than the [6+4] product by 5.2 kcal mol$^{-1}$, due to a combination of a tether effects and steric repulsions between two methyl groups in the [4+2] product. The results strongly support the plausibility of the biosynthetic [6+4] cycloaddition, and may lead to the development of new synthetic strategies for using transannular [6+4] cycloaddition to construct polycyclic scaffolds featuring unbridged 10-membered rings.
1.6 References


(22) We modeled this step and found it to be exergonic by 13.6 kcal mol$^{-1}$.


(40) The truncated model substrate 5t and the two cycloadducts are shown below.


Chapter 2. How Water and Enzyme SpnF Catalyze the Transannular Diels–Alder Reaction: A Study of Medium Effects on Dynamic Mechanisms

2.1 Abstract

Enzymes have been known to catalyze chemical reactions by lowering the reaction barrier. A long-persistent fundamental question is whether enzyme could control reaction mechanism by altering the dynamics of chemical reaction. Here we present an Environment-Perturbed Transition State Sampling method to explore the mechanism of the monofunctional Diels–Alderase SpnF-catalyzed transannular [4+2] cycloaddition (Diels–Alder reaction). Simulations reproduce the measured rate constants of the catalyzed and uncatalyzed reactions. In contrast to the uncatalyzed reaction in water, the enzymatic reaction was revealed to proceed via an ambimodal transition state, which leads to both [4+2] and [6+4] cycloadducts. In particular, enzyme enhances the formation of [4+2] product, with respect to the counterparts in the gas phase, and in water. Our investigation brings us one step closer to ultimately understanding and predicting mechanisms, dynamics, catalytic efficiency and selectivity of enzymatic reactions in molecular detail.

2.2 Introduction

The enzyme SpnF catalyzes the transannular Diels–Alder (DA) reaction shown in Figure 2.1.\textsuperscript{1} We have previously studied this reaction in the gas phase and discovered that the reaction occurs via an ambimodal transition state.\textsuperscript{2} Quantum mechanical calculations and quasi-classical trajectory simulations demonstrated that the reaction pathway involves a bifurcating potential energy surface (PES) after passing through the ambimodal transition state (TS). The spontaneous
reaction of the substrate 1 proceeds via a single TS that leads to both the observed [4+2] adduct 2, and an unobserved [6+4] adduct 3 (Figure 2.1). Adduct 3 can convert into 2 via a Cope rearrangement.

![Diagram](image)

**Figure 2.1.** SpnF-catalyzed transannular Diels–Alder reaction of 1 to form 2. This is a step in the biosynthesis of spinosyn A.

We have now investigated the reaction in water and in the enzyme SpnF. We explored with quantum mechanics/molecular mechanics (QM/MM) methods how water and the enzyme influence the rate of reaction and the product ratio. We have further developed a
Solvent/Enzyme-Perturbed Transition State Sampling method\(^4\) in order to investigate the energetics and reaction dynamics of this cycloaddition reaction in water and in the enzyme.

Despite the wide applications of the Diels–Alder reaction since its discovery in 1928,\(^5,6\) the identification of natural enzymes that have evolved to catalyze Diels–Alder reactions (Diels–Alderases or DAases) is so far limited.\(^7\) This has engendered controversies about the existence of such enzymes, but extensive surveys of secondary metabolites indicate that hundreds of natural products are potentially biosynthesized by DAases.\(^8\) Only a handful of purified enzymes have been demonstrated to catalyze DA reactions, and these enzymes often catalyze other reactions, such as oxidations, thus leaving their specific influence on the DA reactions uncertain.\(^9\)

In 2011, Liu et al. discovered the first monofunctional DAase, SpnF, which solely catalyzes a Diels–Alder reaction in the biosynthetic pathway of spinosyn A. Since then, a handful of new DAases have been characterized by genetic and biochemical assays. However, there were no reports of the 3D structural details of these natural DAases until 2015, when the crystal structure of SpnF was reported.\(^10\) More recently, the crystal structures of two new DAases, Pryl4 and AbyU, have been obtained.\(^11,12\) These provide detailed structural information on DAases, but the mechanisms by which the enzymes catalyze DA reactions are still largely unknown. This may be due in large part to the lack of ionic intermediates or covalent bonding to the enzyme, both of which provide handles for stabilization by enzymes.

Computational simulations have provided structural and energetic insights into mechanisms of numerous enzymatic reactions. Nevertheless, quantitative predictions on the catalytic proficiency and selectivity of enzymatic reactions are still difficult. The recent report of the crystal structure of AbyU and associated QM(SCC-DFTB)/MM(FF14SB) studies by Race et al. have shown that the proposed DA reaction likely proceeds via a concerted, yet highly
asynchronous transition state in the active site of the enzyme AbyU. However, the calculated potential of mean force (PMF) barrier largely underestimates the experimental barrier. The accurate computation of free energies is challenging because it requires both accurate descriptions of interatomic interactions and massive samplings of molecular conformations.

SpnF catalyzes a transannular [4+2] cycloaddition of polyene macrolactone 1 to form 2 (Figure 2.1). The TS is “ambimodal” in the gas phase, which not only gives the observed Diels–Alder adduct, but also a [6+4] adduct. The product distribution of individual molecules reacting on a bifurcating PES is determined by dynamical effects, such as motions and momenta of atoms in the ambimodal TS on a time scale of femtosecond (fs). Reaction trajectory simulations are essential to elucidating the initial distribution (selectivity) of the Diels–Alder and [6+4] adduct.

Ambimodal transition state, and PES bifurcations have been found in many chemical reactions. Post-transition-state dynamics have been employed to explain and predict selectivities of chemical reactions of this type. PES bifurcations in enzymatic reactions are, so far, a rarity. Tantillo et al. proposed the involvement of PES bifurcations in the cationic rearrangements occurring in terpene biosynthesis. Gas-phase reactive trajectory simulations were used to model these reactions. Major et al. supported the existence of such bifurcations in terpene cyclases by PMF calculations with a QM/MM method combined with reactive trajectory simulations initiated from the PMF surface.

How enzymes might influence the dynamical behaviors of bifurcating reactions remains largely unknown. We have developed a Solvent/Enzyme-perturbed Transition State Sampling (S/EPTSS) method for free energy calculations and reaction dynamics simulations in solvents
and enzymes. This enables the study of time-resolved mechanisms of reactions in condensed media, and provides insights into the origins of catalysis in molecular detail.

2.3 Computational Methods

The Solvent/Enzyme-perturbed Transition State Sampling (S/EPTSS) method consists of four major steps. (1) Construction of the reaction/medium models. Initial structures of reactants and transition states were optimized in the gas phase at the M06-2X/6-31G(d) level of theory,\textsuperscript{22} using Gaussian 09.\textsuperscript{23} The optimized structures were then solvated in a water box using AmberTools 14\textsuperscript{24} for the aqueous system. For the reaction in enzyme, the substrate or TS were docked into the enzyme active site using AutoDock Vina. (2) Configuration Sampling. Classical molecular dynamics (MD) were performed using Amber 14 on the substrate and the transition state for 10 ns in water and for 500 ns in enzyme. The FF99SBildn force field was used for protein residues. General Amber Force Field (GAFF) was used for the reactant and transition structures. During the classical MD on the transition state, restraining potentials of 500 kcal/mol/Å\textsuperscript{2} were applied to the reaction coordinates in the transition state. Snapshots (typically 100) of reactant and TS are sampled from production trajectories with 5 ps intervals in water and 1 ns intervals in enzyme. (3) Free energy calculations. Optimization and frequency analysis were performed at the QM/MM level on the sampled snapshots. M06-2X/6-31G(d) was used for the QM calculations. The free energy of each configuration was calculated by adding the QM energy, QM thermal correction energy, and QM/MM interaction energy. No intra-solvent or intra-enzyme energies were included; we assume they achieved average equilibration energy in each snapshot, but this does introduce some random error. The free energy activation barriers were calculated as the difference between the free energy averages of the TS and the free energy
of the reactant ensembles in solvent or enzyme. (4) Reaction dynamics. Reactive trajectory simulations were initiated from the random normal mode sampled transition states in water or in enzyme. Normal mode sampling was conducted at 300K for each sampled TS structure to obtain coordinates and momenta in a quasiclassical manner. These trajectories were propagated forward and backward for 150 fs each.

2.4 Results and Discussion

2.4.1 Conformational ensembles for reactant, ambimodal TS-A and Diels-Alder TS-B

The substrate in the SpnF-catalyzed Diels–Alder reaction is a 22-membered polyene lactone. Due to its conformational flexibility, we performed classical MD simulations on the substrate in water and in the enzyme. An ensemble of reactants was built up by taking 100 snapshots of the substrate, each 100 picoseconds (ps) in water, or each nanosecond (ns) in the enzyme. As shown in Figure 2.2, the reactant does have some backbone flexibility, but this consists mainly of two typical conformations that we call R-A and R-B. In the gas phase, the lowest energy conformer is R-A, which possesses an intramolecular hydrogen bond (H-bond). Conformer R-B is 5.2 kcal/mol higher in energy. In water, R-B is stabilized relative to R-A by H-bonds with water molecules. R-B is stabilized in the enzyme by hydrogen bonds with enzyme residues. R-B is less than 1% at equilibrium in the gas phase, 80% in water and 80% in enzyme. In either R-A or R-B, the reacting diene adopts a lowest energy s-trans conformation, which will undergo a conformational change to the reactive s-cis conformation in the transition state.
Ensembles of reactants from classical MD in water and in the enzyme. Water molecules and enzyme residues are not displayed. R-A and R-B are two representative conformations optimized in the gas phase. The dihedral angle used for discriminating between the two conformations is highlighted.

Two corresponding conformers of the transition states (ambimodal TS-A and Diels–Alder TS-B) were also found (Figure 2.3). The ambimodal TS-A is preferred over the Diels–Alder TS-B by 3.3 kcal/mol in the gas phase. For water and the enzyme, TS-MD simulations were conducted separately on both TS conformers. As noted, ensembles for ambimodal TS-A and for Diels–Alder TS-B were constructed by taking 100 snapshots of the solvent box with a 100 ps interval in water and a 1 ns interval in the enzyme. For each ensemble, subsequent QM/MM calculations were conducted to optimize the reactant, ambimodal TS-A or Diels–Alder TS-B in the snapshots, and to compute the free energies by averaging.
Figure 2.3. Ensembles of ambimodal TS-A and Diels-Alder TS-B in enzyme. Ambimodal TS-A and Diels-Alder TS-B are representative conformations for their corresponding ensembles. The dihedral angle used for discriminating two conformations is highlighted. The intramolecular H-bond is the first case stabilizes a conformation suitable for the ambimodal TS-A.

2.4.2 Computed free energies of activation

Figure 2.4 shows the averaged free energy barriers for ambimodal TS-A and Diels–Alder TS-B in water and in the enzyme. The values are averages for different numbers of snapshots. The averages based on a small number of snapshots have large standard errors. The standard errors decrease, and the energetics converge after including a large number of snapshots. This confirms the need for a sufficient conformational sampling for the free energy calculations of reactions in condensed media.26,27
Figure 2.4. Free energy barriers for the cycloaddition reaction of 1 in water and in the enzyme. Each blue or red dot represents an average of free energies for ambimodal TS-A or Diels-Alder TS-B under the corresponding number of snapshots in water or in enzyme. The standard error is shown for each average. The barriers labeled on the graphs are the averages of free energies with 100 snapshots. Values are in kcal/mol, for 25 °C and 1 M.

<table>
<thead>
<tr>
<th></th>
<th>Barrier for ambimodal TS (kcal/mol)</th>
<th>Barrier for Diels-Alder TS (kcal/mol)</th>
<th>Experiment (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>27.8±1.9</td>
<td>22.9±2.1</td>
<td>22.0±0.01</td>
</tr>
<tr>
<td>Enzyme</td>
<td>18.8±1.0</td>
<td>23.4±1.1</td>
<td>18.3±0.1</td>
</tr>
</tbody>
</table>

The Diels–Alder TS-B is preferred in water, while the ambimodal TS-A is favored in the enzyme. The computed barriers are consistent with the experimental values of 22.0 ± 0.01 kcal/mol in water and 18.3 ± 0.1 kcal/mol in SpnF, calculated from rates measured at 25 °C.

To elucidate the origin of catalysis, we analyzed hydrogen bonding in reactants, ambimodal TS-A, and Diels-Alder TS-B ensembles after QM/MM optimizations. In water, the Diels-Alder TS-B does not involve an intramolecular H-bond between the hydroxyl and the carbonyl group, and R-B and the Diels-alder TS-B are favored. This enables H-bonds to
surrounding water molecules. Statistically, each Diels-Alder TS-B forms 0.4 more H-bonds than the ambimodal TS-A. The reactant involves a mixture of conformations with and without intramolecular H-bonds, although the latter is favored. From reactant to ambimodal TS-A, the number of H-bonds to water decreases, while from reactant to Diels-Alder TS-B, the number of H-bonds increases. This causes the favorable barrier for Diels-Alder TS-B.

In the enzyme, both intra- and intermolecular H-bonds are observed. The hydrogen bonds measured in the reactant and two conformers of the TS are shown in Table 2.1. H-Bonds between substrate and H42, E152 (to the TS), A252 (to the reactant) stabilize the binding configuration of the TS and the reactant. Two typical snapshots are also shown for ambimodal TS-A and Diels-Alder TS-B with H-bonding to their surrounding residues. H-Bonds between the residues and the highlighted C=O, which conjugates to the diene, can decrease the energy barrier of the cycloadditions.28

Table 2.1. Percentage of structures that have H-bond interactions with enzyme residues in ensembles of Reactant, TS-A, and TS-B after QM/MM optimizations. An H-bond is defined as having H–O bond length shorter than 2.25 Å and the O–H–O bond angle greater than 150°.
One hundred snapshots after QM/MM optimization were selected in each ensemble for the analysis. H-bonds to W256 are present in 61% of the reactant snapshots, and in 86% and 87% of the ambimodal TS and Diels-Alder TS, respectively. From reactant to TS, the ambimodal TS-A experiences an enhancement of H-bond c (intramolecular H-bond) from 9% to 99%, while Diels-Alder TS-B shows a moderate increase in H-bond b (to T195) percentage from 0% to 21%. This explains in part why the ambimodal TS is more favorable in the enzyme. In addition, the protein pocket is highly hydrophobic, and no water molecules are detected within 4 Å of the mass center TS during the MD. The Diels-Alder TS-B in the enzyme cannot be stabilized by intermolecular H-bonds provided by water or protein residues.

2.4.3 Reaction dynamics simulations

Enzymes can bind reactants and transition states by electrostatic and hydrophobic interactions. These interactions may favor certain transition state geometries, and influence reaction dynamics as well.

SpnF presents an intriguing case in which the ambimodal TS-A is favored in the gas phase and in enzyme, while the Diels-Alder TS-B geometry is favored in water. We have probed how different TS geometries are favored in different media, and how the ambimodal TS-A geometries are altered by enzyme relative to water and the gas phase. The possible role of dynamics on catalysis by enzyme has been a significant topic of discussion ever since Wright and Benkovic reported correlations between dynamics motions of remote residues and alternation of catalytic pathway by mutation of these residues. After considerable debate, the current consensus is that relatively slow, ms-μs, motions loops and remote residues do indeed
alter the structure of the active site, but fast ps-fs coupling of vibrational motions to motions along the reaction coordinate have a mere influence on the rate of reactions.\textsuperscript{30} We note that residues in contact with the reactant as they vibrate through the transition zone can influence energetics, but the motion of which residues towards and away from the transition state are much slower than the reaction event.

Figure 2.5 shows the distribution of sampled geometries for Diels-Alder TS-B in water (a), and ambimodal TS-A in the gas phase (b), in water (c) and in enzyme (d). Bonds 2 and 3 are formed in the [4+2], and the [6+4] adducts, respectively. For Diels-Alder TS-B in water, the distribution of bond 2 is 2.62±0.42 Å, and that of bond 3 is 3.65±0.51 Å. The two bonds on average differ by ~1 Å. The formation of the [4+2] adduct is almost always observed in dynamics from this TS (see later). In contrast, the ambimodal TS-A in enzyme shows that bonds 2 and 3 are much closer in length with 2.96±0.25 Å for bond 2, and 3.12±0.26 Å for bond 3. In the gas phase, the difference between bonds 2 and 3 is further narrowed, making the formation of [6+4] adduct more likely. These results indicate that the nature of the TS varies in the gas, water, and enzyme environment. This is a result of the average dynamical environment and alters the pathway that each trajectory must take to achieve productive formation of product. In this case, the protein (or water) and reaction dynamics are coupled, but on an average, not fs timescale.
Figure 2.5. Distribution of bonds 2 and 3 in 240 transition state geometries for (a) Diels-Alder TS-B in water, (b) ambimodal-A TS in the gas phase, (c) ambimodal TS-A in water, and (d) ambimodal TS-A in the enzyme. Bond 2 in blue leads to the [4+2] adduct, while bond 3 in red leads to the [6+4] adduct. For (a), (c), and (d), transition state geometries were sampled by using normal mode sampling on 60 transition structures optimized in various snapshots of enzyme or of water.

Reaction dynamics trajectories were initiated from transition state geometries after normal mode sampling to explore the dynamics of product formation. Each reactive trajectory was propagated for 300 fs using the QM/MM method.
Figure 2.6 displays typical trajectories propagated in water and in the enzyme. The third and fourth panels in each row indicate the time at which bond 1 and either bonds 2 or 3 are formed, defined as achieving a distance of 1.6 Å. (Bonds 1, 2, and 3 are labeled in Figure 2.5.) Bond 1 forms in both adducts. Bond 2 forms in the [4+2] adduct, while bond 3 forms in the [6+4] adduct. Figure 2.6a and Figure 2.6b show the production of [4+2] adduct and [6+4] adduct, respectively. In Figure 2.6a, bond 1 forms at 30 fs, then bond 2 forms at 90 fs. In Figure 2.6b, bond 1 forms at 18 fs, then bond 3 forms at 42 fs. Likewise, Figure 2.6c and Figure 2.6d represents the formation of [4+2] adduct and [6+4] adduct, respectively, in the enzyme, with two bonds forming at 36 fs and 86 fs for the formation of the [4+2] adduct, and at 67 fs and 138 fs for the formation of the [6+4] adduct.

**Figure 2.6.** Typical trajectories for the formation of (a) [4+2] adduct in water, (b) [6+4] adduct in water, (c) [4+2] adduct in enzyme, and (d) [6+4] adduct in enzyme. We define 1.6 Å as the criterion for C-C bond formation.
Figure 2.7 shows overlays of 100 trajectories plotted according to lengths of forming bonds 1, 2, and 3. The ratios of [4+2]:[6+4] products are also presented. In water, the Diels-Alder TS leads almost exclusively to Diels-Alder adduct, with only one out of 100 trajectories leading to a [6+4] adduct. For trajectories passing through the ambimodal TS, bifurcation is observed with a [4+2]:[6+4] ratio of 3.5:1 in enzyme, 1.6:1 in water, and 1:1.1 in the gas phase. The selectivity for [4+2] and [6+4] adduct results from the competition between the formation of bond 2 and bond 3. From gas phase to water, a preference towards [4+2] adduct was observed, even though the distributions of the transition state geometries for ambimodal TS-A are very similar in both media. The dipole moment of the [4+2] adduct is 4.74 Debye, while that of the [6+4] adduct is 3.46 Debye. This indicates that [4+2] adduct is better stabilized by polar solvent. In addition, trajectories were propagated in implicit solvents (polarizable continuum model\textsuperscript{31}) to explore how the polarity of the medium influences the product distribution. The [4+2]:[6+4] ratio is 1:1 in implicit hexane, and 1.5:1 in implicit water. This further supports that increase in solvent polarity promotes the formation of the [4+2] adduct over the [6+4] adduct.
Figure 2.7. Distributions of reactive trajectories initiated from Diels-Alder TS in water, ambimodal TS in the gas phase, in water, and in enzyme. One hundred randomly chosen trajectories were plotted in each case. Trajectories leading to [4+2] adduct are shown in blue, and those leading to [6+4] adduct are shown in red.

To investigate the influence of the enzyme on the transition state, we also propagated the trajectories with mechanical embedding only, which removes the electrostatic interaction between the reacting molecule and its surrounding protein residues. In this case, the [4+2]/[6+4] ratio of trajectories is 2.8:1, maintaining most of the preference for the [4+2] adduct. This indicates that electrostatic interactions favor slightly the formation of the [4+2] adduct, while VDW interactions between the reacting molecule and the hydrophobic residues steer the trajectories towards the [4+2] adducts in dynamics.
We further investigated how hydrophobic residues favors the formation of the \([4+2]\) adduct. Classical MD simulations show that there are close interactions between enzyme alkyl residues, leucine and valine, and the atoms C12 and C14 that form bond 2. L198 is close to both C12 and C14. V26 is near C2 and C4 (Figure 2.8). We analyzed on the distances from the hydrophobic residues to each of the four carbons, as well as the angle between residue H-C2-C14 and residue H-C4-C12. We expect that an angle near 180° implies that dynamic vibrations of the H may force the C-C bonds closer. As represented in the distribution of distances shown in Figure 2.8, V26 is closer to C4 than C2. The average distance to C4 is 3.60 Å, with 7.0% of the distances smaller than 2.9 Å (the sum of VDW radius of C and H). The average distance to C2 is 4.07 Å, with 0.4% smaller than 2.9 Å. V26 keep a more linear alignment with C4-C12, with the average R-C4-C12 angle of 151.0°. The average R-C2-C14 angle is 124.0°. L198, on the other hand, is in almost identical contact with C14 and C12. Statistically, the hydrophobic contacts from SpnF constrain C4 and C12 to be closer, leading to higher probability for bond formation in the \([4+2]\) reaction. This is reflected in the relatively larger difference of lengths of partial bond 2 and 3 in the ambimodal TS in the enzyme compared to the gas phase and in water.
2.5 Conclusion

We have developed the Solvent/Enzyme-Perturbed Transition State Sampling (S/EPTSS) method, and applied it to computations of free energy barriers and reaction dynamics of reactions in solvent water and in the enzyme SpnF. Using this method, we investigated the medium effects and dynamic mechanisms of SpnF-catalyzed transannular Diels–Alder reactions. Two distinct transition states were found for the reaction in water and in the enzyme. The computed reaction barriers are in good agreement with the experimentally measured rates of reaction. Important residues that contribute to the overall catalytic process and product distributions were identified. Our study provides insights into how water and enzyme residues influence the rates of reaction and the distributions of products in a dynamically controlled ambimodal reaction mechanism.
2.6 Reference


(20) Hong, Y. J.; Tantillo, D. J. Nat. Chem. 2014, 6, 104.


Cycloadditions of Tropone to Dimethylfulvene

3.1 Abstract
The mechanisms and selectivities of the cycloadditions of tropone to dimethylfulvene have been investigated with M06-2X and B3LYP-D3 density functional theory calculations and molecular dynamics simulations. The originally proposed reaction mechanism (Houk) involves a highly peri-, regio- and stereoselective [6_F+4_T] cycloaddition of tropone [4π] to dimethylfulvene [6π], followed by a [1,5] sigmatropic hydrogen shift, and finally, a second [6+4] cycloaddition of tropone [6π] to the cyclopentadiene moiety [4π]. An alternative mechanism was proposed by Paddon-Row and Warrener: the initial cycloaddition involved a different [6_T+4_F] cycloaddition in which fulvene acts as the 4π component, and a subsequent Cope rearrangement produces the formal [6_F+4_T] adduct. We now demonstrate that the initial cycloaddition involves an ambimodal transition state that lead to both the proposed [6+4] adducts. These adducts can interconvert through a [3,3] sigmatropic shift (Cope rearrangement). Molecular dynamics simulations reveal the initial distribution of products and provide insights into the time-resolved mechanism of this ambimodal cycloaddition. Competing [4+2] cycloadditions and various sigmatropic shifts are also explored.

3.2 Introduction
Diels–Alder reaction and 1,3-dipolar cycloaddition involve six π electrons. Cycloaddition reactions involving more than six π electrons are termed higher-order cycloadditions. As a prototype higher-order cycloaddition, the [6+4] cycloaddition\(^1\) was first predicted to be an orbital
symmetry-allowed pericyclic reaction under thermal conditions in 1965 by Woodward and Hoffmann (Scheme 3.1). At that time, R. B. Woodward proposed that one of us search for such a cycloaddition. One year later, Cookson and Ito independently reported the reaction of tropone with cyclopentadiene to afford the exo [6+4] cycloadduct selectively (Scheme 3.1b). In the 50 years since then, various research groups have explored and developed this reaction. Garst and Rigby have put this reaction to synthetic use, but its synthetic applications remain limited. A major drawback is that there are many potential competing reactions including other allowed pericyclic processes such as Diels–Alder reactions. Low periselectivity directly leads to relatively low yields of the desired cycloadducts.

The origins of periselectivity of higher-order cycloadditions are poorly understood. We have recently discovered the involvement of intramolecular (transannular) [6+4] cycloadditions in the biosynthesis of natural products (Scheme 3.1, c and d). These cycloadditions involve ambimodal transition states (a transition state leading to different products). In such cases post-transition-state dynamics are important for controlling the periselectivities.
Scheme 3.1. Representative [6+4] cycloadditions

There is only one example of a catalytic enantioselective [6+4] cycloaddition in the literature prior to 2017: a chiral titanium (IV) Lewis acid catalyzes an intramolecular [6+4] cycloaddition with only moderate enantioselectivity (Scheme 3.1e). Most recently, Jørgensen and coworkers reported the first catalytic enantioselective intermolecular [6+4] cycloaddition with excellent enantioselectivity, using a chiral primary amine catalyst to activate 2-cyclopentenone (Scheme 3.1f). Besides [6+4] cycloadditions, the authors also reported [8+2] and formal [4+2] cycloadditions. They found that the periselectivities are controlled based on factors such as the ring size of the cycloalkenones and the substitution patterns.
In 1967, Houk discovered the 2:1 adduct of tropone to dimethylfulvene. This was reported first at a Woodward Thursday night group meeting, but the structure of the adduct was not fully established with 60 MHz NMR. In 1970, at Louisiana State University, with the help of master NMR spectroscopist Norman Bhacca, Houk reported the structure of this novel double [6+4] cycloadduct (Scheme 3.2). A complex pentacyclic molecule (3) was formed with remarkably high selectivity by the reaction of tropone (1) with dimethylfulvene (2). When the reaction was carried out at room temperature for two weeks, an intermediate (4) and a Diels–Alder product (5) were also observed (Scheme 3.2a).

**Scheme 3.2.** The double [6+4] cycloaddition of tropone to dimethylfulvene

The mechanism proposed originally is shown in Scheme 3.2b. Tropone (1) and dimethylfulvene (2) undergo an initial [6+4] cycloaddition to form cycloadduct 6. The cyclopentadiene ring in 6 undergoes a [1,5] sigmatropic hydrogen shifts to form 4, which
contains a more substituted alkene and is thus more stable. Finally, 4 reacts with another molecule of tropone (1) in a second exo [6+4] cycloaddition to afford compound 3. The initial [6+4] cycloaddition that forms 6 represents the first example of fulvene serving as a 6π component.

Shortly after, Paddon-Row and Warrener elaborated upon the originally proposed mechanism (Figure 3.1, pathway 1). They proposed that the formation of 6 involves a [6_{r}+4_{F}] cycloaddition to form 7, where tropone is the 6π electron addend. Then 7 could convert into 6 by a Cope rearrangement (Figure 3.1, pathway 2). One piece of the evidence for this proposal was the identification of the analogous compound 7’ in the reaction of tropone with an isobenzofulvene. In addition, extended Hückel MO calculations supported the formation of 7 over 6. However, no direct evidence could be obtained at that time to exclude the possibility of pathway 1, because 7 was never detected in the reaction mixture.

![Figure 3.1. Proposed mechanisms of the initial [6+4] cycloaddition of tropone to dimethylfulvene.](image-url)
The highly peri-, regio- and stereoselective cycloaddition of tropone to dimethylfulvene renders this reaction an interesting platform upon which to investigate origins of periselectivity in higher-order cycloaddition reactions. We have now used modern density functional theory calculations to explore the whole reaction pathway, with a focus on the selectivities of the first [6+4] cycloaddition.

3.3 Computational Methods

All density functional theory (DFT) calculations were performed using Gaussian 09.14 Geometry optimizations and frequency calculations were performed at the B3LYP/6-31G(d) level of theory,15 with Grimme’s D3 empirical dispersion correction.16 Normal vibrational mode analysis confirmed that optimized structures are minima or transition structures. Truhlar’s quasiharmonic correction was used to compute molecular entropies to reduce error caused by the breakdown of the harmonic approximation, by setting all positive frequencies that are less than 100 cm$^{-1}$ to 100 cm$^{-1}$.17 Since the M06-2X functional18 has been shown to yield more accurate energetics for cycloaddition reactions,19 M06-2X/6-311+G(d,p) single-point energies were computed on the B3LYP-D3-optimized structures. Solvent effects were evaluated with the polarizable conductor model (PCM).20 All 3D renderings of stationary points were generated using CYLview.21 GaussView22 and Avogadro23 were used to construct initial structures used in our computations.

Molecular dynamics (MD) simulations were performed at the B3LYP-D3/6-31G(d) level of theory in the gas phase. Quasiclassical trajectories (QCTs) were initiated in the region of the potential energy surface near TS-1. Normal mode sampling involved adding zero-point energy for each real normal mode, and performing a Boltzmann sampling of geometries to afford the
thermal energy available at 298 K with a random phase. The trajectories were then propagated forward and backward, 500 fs in each direction. The classical equations of motion were integrated with a velocity-Verlet algorithm using Singleton’s program Progdyn, with the energies and derivatives computed on the fly with B3LYP-D3 using Gaussian 09. The step length for integration was 1 fs.

3.4 Results and Discussion

3.4.1 Possible [6+4] and [4+2] cycloadducts

The possible allowed thermal cycloadditions are listed in Figure 3.2. The corresponding cycloadducts and computed free energies of reaction are also shown. Carbonyl additions and the possibility of fulvene acting as an s-trans diene are excluded.
Figure 3.2. Possible allowed thermal cycloadditions we computed here, with the corresponding cycloadducts and computed free energies of reaction. Carbonyl additions and the possibility of
fulvene acting as an $s$-$trans$ diene are excluded. Free energies are computed at the PCM(diethylether)/M06-2X/6-311+G(d,p)//B3LYP-D3/6-31G(d) level and are in kcal/mol.

In the [6+4] cycloadditions, fulvene could behave as the 6π electron addend (Figure 3.2a), or as the 4π electron addend (Figure 3.2b). Analogously, in the [4+2] cycloadditions, fulvene could serve as the 2π (Figure 3.2c) or the 4π (Figure 3.2d) component. For each mode of cycloaddition, there are four different cycloadducts (only two for 6T+4F due to symmetry), originating from different regio- (syn/anti) or stereo- (endo/exo) approaches of the reactants. These add up to 14 different cycloadducts.

The originally proposed adduct (6) from the 6T+4F cycloaddition (Scheme 3.2, pathway 1) is relatively stable ($\Delta G_{\text{rxn}} = -3.0$ kcal/mol). However, its regioisomer 6b is almost isoenergetic ($\Delta G_{\text{rxn}} = -3.1$ kcal/mol). The Paddon-Row–Warrener proposed intermediate (7) (Scheme 3.2, pathway 2) is slightly higher in energy than the reactants ($\Delta G_{\text{rxn}} = 1.3$ kcal/mol). Thus, it could be generated in the reaction mixture and then convert into the more stable adduct 6, as suggested by Paddon-Row and Warrener. Adduct 7a is much higher in energy than 7, in agreement with the generally observed exo selectivity of [6+4] cycloadditions.

The 4T+2F cycloadditions (Figure 3.2c) are favored most thermodynamically. The most stable product 5 ($\Delta G_{\text{rxn}} = -7.2$ kcal/mol) corresponds to an adduct that was observed experimentally in 4% yield. The Diels–Alder adducts from the alternative 4F+2T cycloadditions (Figure 3.2d) are much higher in energy (~14 kcal/mol) than the reactants, and are unlikely to form in the reaction.

3.4.2 Transition states and free energies of activation
Figure 3.3 shows the transition structures (TS) we successfully located starting from the 14 cycloadducts and stretching the bonds that had formed. Interestingly, we were only able to find 10 distinct TSs. They are listed and numbered in ascending order of relative free energies (TS-1–10).

Figure 3.3. Transition structures (TS) that lead to different cycloadducts. Free energies of activation are computed at the PCM(diethylether)/M06-2X/6-311+G(d,p)//B3LYP-D3/6-31G(d) level and are in kcal/mol.
In the cases of the first four transition structures, **TS-1–4**, the searches for TSs for different cycloadducts resulted in only one transition structure. For example, the searches for transition structures for adduct 6 and 7 both led to **TS-1**. These four TSs are highly asynchronous; the shortest partially formed σ bonds are ~1.9 Å, and the second and third partially formed σ bonds are much longer at ~3 Å. For each TS, the shortest forming bond corresponds to the one formed in both adducts, while the formation of either the second or third bond determines which one of the two distinct adducts is formed.

Figure 3.4 illustrates this phenomenon by highlighting (in green) the atoms involved in the two different modes of cycloaddition for the lowest energy transition state **TS-1** ($\Delta G^\ddagger = 27.9$ kcal/mol). Adduct 7 is generated when bonds C2-C15 and C3-C13 are formed, while the formation of bonds C2-C15 and C7-C24 leads to adduct 6. The less stable adduct 7 could convert into the more stable adduct 6 by a [3,3] sigmatropic shift (Cope rearrangement) via **TS-Cope-1**, which has a barrier of 26.3 kcal/mol. Curiously, the later resembles 6 more than 7, in contrast to the Hammond’s postulate prediction.
Figure 3.4. Ambimodal transition state $\text{TS-1}$ that could lead to either 7 or 6. A Cope rearrangement converts 7 into 6 via $\text{TS-Cope-1}$. Free energies are in kcal/mol.

A transition state that leads to multiple products has been termed “bis-pericyclic”$^{25a}$ or “ambimodal”$^{25d}$. The occurrence of such a transition state is a result of a potential energy surface (PES) bifurcation.$^{26}$ The phenomenon of PES bifurcation was discovered for cycloadditions by Caramella$^{25a}$ and has been found frequently since then.$^{25}$ It has been proposed in many chemical reactions such as the cationic rearrangements in terpene biosynthesis.$^{27}$ We have recently demonstrated the involvement of intramolecular (transannular) $[6+4]/[4+2]$ ambimodal cycloadditions in the biosynthesis of heronamides and spinosyn A.$^8$ The reaction between tropone and dimethylfulvene provides a new example of this type, a $[6+4]/[4+6]$ ambimodal cycloaddition. Thus, in contrast to kinetic control, the initial distribution of adducts 7 and 6 is instead determined by post-transition-state dynamics.$^{28}$
3.4.3 Molecular dynamics simulations

Direct chemical dynamics simulations, which integrate the technologies of classical dynamics and ab initio electronic structure theory, provides the ideal tool to study reactions involving PES bifurcations and ambimodal transition states. MD simulations predict the distribution of products for bifurcating reactions, and provide valuable information on the time-resolved mechanisms. Figure 3.5 shows snapshots of two representative reactive trajectories, which lead to (a) adduct 7 and (b) adduct 6, respectively.

![Snapshots of reactive trajectories](image)

**Figure 3.5.** Snapshots for two typical reactive trajectories of tropone-dimethyl fulvene cycloaddition that lead to (a) adduct 7 and (b) adduct 6, respectively. Snapshots at 0 fs correspond to transition state geometries where trajectories are initiated.
In Figure 3.5a, the two reactants are about 3.5 Å away from each other at –96 fs (0 fs corresponds to the transition state geometry where trajectories are initiated). After they pass the transition state at 0 fs, it takes 34 fs for the first bond to form (defined by C-C bond length shorter than 1.6 Å). The second bond forms at 94 fs and adduct 7 is formed. The time gap between the formation of the two bonds is 60 fs, at the borderline between a dynamically concerted and a dynamically stepwise mechanism.30 Analogously, some trajectories lead to adduct 6, as represented by the one shown in Figure 3.5b. In this case, after the formation of the same first bond at 47 fs, a different second bond forms at 132 fs.

Out of the 117 reactive trajectories we obtained in our simulations, 107 trajectories (91%) go to adduct 7, while 10 trajectories (9%) lead to adduct 6 (Shown in Figure 3.6). The results suggest that both adducts are formed initially in the reaction, but there is a dynamic preference (10:1) for the formation of 7 over 6.
Figure 3.6. Distribution of 117 reactive trajectories; 107 (91%) of which lead to 7, and 10 (9%) of which lead to 6. The three axes represent the forming bond lengths and each trajectory has a different color.

3.4.4 Frontier molecular orbital (FMO) analysis

We have also performed FMO calculations to investigate the influence of orbital interactions on the selectivity of reaction. The results are shown in Figure 3.7. The computed FMO energies suggest that the main orbital interaction comes from the overlap between the LUMO of tropone (1.5 eV) and the HOMO of fulvene (−7.9 eV). As shown in Figure 3.7, the HOMO of fulvene has negligible coefficients on the exocyclic carbon atom (C24). Thus, FMO analysis predicts that the formation of 7 is preferred than 6, to maximize orbital interactions in the transition state. This is consistent with the fact that in TS-1 (Figure 3.4), the forming bond length of C7-C24 (3.20 Å) is longer than that of C3-C13 (2.81 Å).
Figure 3.7. Computed HOMO and LUMO of tropone and dimethylfulvene, respectively, at the HF/6-31G(d)//B3LYP-D3/6-31G(d) level.

3.4.5 [1,5] Hydrogen shift and second [6+4] cycloaddition of tropone

When adduct 6 was formed during the reaction, whether directly from the cycloaddition of tropone to fulvene, or through the Cope rearrangement of 7, it never exceeded 10% of the reaction mixture as monitored by NMR. The original hypothesis is that it undergoes rapid [1,5] sigmatropic hydrogen shift to afford 4 (isolated in 22% yield). We explored this process and found that the barrier for this [1,5] shift is only 23.7 kcal/mol (Figure 3.8, TS-11). The cyclopentadiene 4 with more substituted alkene is more stable than 6 by 2.1 kcal/mol. Compound 4 could then undergo another exo-selective [6+4] cycloaddition with tropone via TS-12 ($\Delta G^* = 28.3$ kcal/mol) to form the final product 3, which is in a thermodynamic sink ($-9.5$ kcal/mol) on the PES.
Figure 3.8. [1,5] Sigmatropic hydrogen shifts and the second [6+4] cycloaddition. Free energies are computed at the PCM(diethylether)/M06-2X/6-311+G(d,p)//B3LYP-D3/6-31G(d) level and are in kcal/mol.

Experimentally, when 4 was heated at 50 °C, partial conversion to a new isomer (33% at equilibrium) was observed. Although the new isomer could not be separated from 4, its structure was nevertheless proposed (Figure 3.8, 4a). It was hypothesized that “the cyclopentadiene ring in 4 underwent two reversible [1,5] sigmatropic hydrogen shifts to form 4a".\(^{11}\) We have now optimized the structure of 4a and found that indeed it is slightly higher in energy than 4 by 0.5 kcal/mol. This predicts a 2:1 ratio between 4 and 4a at equilibrium, in excellent agreement with the experiments.\(^{11}\)

The complete reaction profile is shown in Figure 3.9. Tropone (1) and dimethylfulvene (2) undergo a highly peri-, regio- and stereoselective and ambimodal [6+4]/[4+6] cycloaddition via TS-I to form adducts 6 and 7 simultaneously. 7 then converts into 6, which is thermodynamically more stable, via a Cope rearrangement transition state TS-Cope-1. The cyclopentadiene ring in 6 undergoes rapid [1,5] sigmatropic hydrogen shifts via TS-11 to form 4,
which contains more substituted alkenes and is thus more stable. Finally, 4 reacts with another tropone in an exo-selective [6+4] cycloaddition via TS-12 to afford a pentacyclic compound 3.

![Diagram of reaction pathway]

**Figure 3.9.** Complete reaction pathway. Free energies are computed at the PCM(diethylether)/M06-2X/6-311+G(d,p)//B3LYP-D3/6-31G(d) level and are in kcal/mol.

### 3.5 Conclusion

We have explored the mechanisms and selectivities of the double [6+4] cycloaddition of tropone to dimethylfulvene, which was first reported in 1970. The two proposed pathways by which the key intermediate 6 are formed are united through the discovery of an ambimodal [6+4]/[4+6] transition state using density functional theory calculations. Molecular dynamics and reaction trajectory simulations provide insights into the time of bond formation for this ambimodal cycloaddition. Reaction dynamics also predict the initial distribution of the two adducts. We have also investigated the proposed [3,3] sigmatropic carbon shift (Cope
rearrangement), [1,5] sigmatropic hydrogen shifts, and a second [6+4] cycloaddition of tropone involved in the whole reaction pathway. The results are in good agreement with the original proposal for the formation of the pentacyclic final product. Our findings establish the origins of this highly peri-, regio- and stereoselective [6+4] cycloaddition. The ambimodal and dynamics-controlled nature of this reaction suggests the potential generality of these phenomena in other higher-order cycloadditions. Understanding how substituents, catalysts, and solvents influence the dynamics and selectivities of higher-order cycloadditions will be the subject of future investigations.
3.6 References


Chapter 4. Biochemical Characterization of a Eukaryotic Decalin-Forming Diels–Alderase

4.1 Abstract

The trans-decalin structure formed by intramolecular Diels–Alder cycloaddition is widely present among bioactive natural products isolated from fungi. We elucidated the concise three-enzyme biosynthetic pathway of the cytotoxic myceliothermophin and biochemically characterized the Diels–Alderase that catalyzes the formation of trans-decalin from an acyclic substrate. Computational studies of the reaction mechanism rationalize both the substrate and stereoselectivity of the enzyme.

4.2 Introduction

Cycloaddition reactions such as intramolecular Diels–Alder (IMDA) reactions are extremely important and versatile synthetic transformations that allow the construction of multicyclic scaffolds with stereocontrol and atom economy.\(^1\) Nature has evolved enzymes to catalyze \([4+2]\) reactions in natural product biosynthetic pathways.\(^2\) Especially from bacterial pathways, a few so-called “Diels–Alderases” (DAases) have been discovered and characterized, including those from the spinosyn,\(^3\) solanapyrone,\(^4\) tetronate/tetramate-containing compound,\(^5\) thiopeptide,\(^6\) and abyssomicin\(^7\) pathways. Structural studies suggest that while there are no conserved sequences for DAases, these enzymes provide an active-site environment that accelerates or enables the cycloaddition reactions to occur.\(^7,8\) The diversity of DAases found to date inspires the discovery of additional enzymes that can catalyze such reactions, which can enable genome mining of new natural products that contain structural features derived from cycloaddition reactions.
IMDA reactions are also proposed to occur widely among fungal natural product pathways, in particular those of polyketide and polyketide–nonribosomal peptide natural products. For example, both the trans-decalin and isoindolone ring systems found in lovastatin\textsuperscript{10} and cytochalasans\textsuperscript{11}, respectively, are thought to derive from cycloaddition reactions of acyclic precursors. Decalin-containing fungal polyketides constitute a large class of natural products with a diverse array of biological activities (Figure 4.1). Biosynthesis of the acyclic substrates that contain both the dienophile and the diene are proposed to be catalyzed by the iterative functions of the highly reducing polyketide synthases (HR-PKSs). While bioinformatic analysis and genetic evidence have suggested that a class of lipocalin-like enzymes may be involved in the formation of the decalin ring systems of Sch210972\textsuperscript{12} and equisetin,\textsuperscript{13} no direct biochemical evidence of enzyme-catalyzed IMDA reactions has been described. This is in part due to the inability to capture an acyclic substrate required for activity verification. In this work, we provide biochemical confirmation of DAase activity from a fungal polyketide–nonribosomal peptide biosynthetic pathway using the biosynthesis of myceliothermophin as a model system.
Figure 4.1. Structures of decalin-containing fungal polyketides and polyketide–amino acid hybrids.

Myceliothermophins, including myceliothermophin E (1) and A (2), are cytotoxic compounds isolated from the thermophilic fungus *Myceliophthora thermophile* (Figure 4.1).\(^{14}\) 1 exhibits IC\(_{50}\) values of <100 nM toward a variety of cancer cell lines. Multiple total syntheses of myceliothermophins have been accomplished to establish the absolute stereochemistry as shown in Figure 4.1.\(^{15,16}\) Both 1 and 2 contain a trans-fused decalin ring system connected to a conjugated 3-pyrrolin-2-one moiety, with 1 containing an exocyclic methylpropylidene unit derived from leucine at C21, while 2 is substituted with a hydroxyl group at the same position. Despite the wide occurrence of the 3-pyrrolin-2-one ring system in natural products,\(^{17}\) its formation has not been widely studied. It is proposed to derive from a Knoevenagel condensation between the \(\alpha\)-carbon (C19) and a reductively released amino aldehyde (C20). This differs from the tetramic acid rings found in equisetin and Sch210972, which arise from Dieckmann cyclization of aminoacylated polyketides.\(^{18}\)
4.3 Results and Discussion

To investigate the biosynthetic pathway of 1 and 2, the genome of the producing organism *M. thermophile* ATCC 42462 was queried for the presence of polyketide synthase–nonribosomal peptide synthetase (PKS-NRPS)-containing genes. Two PKS-NRPS genes were found, and analysis of the neighboring genes indicated that MYCTH _78013 (named mycA), which is near the end of a chromosome, is a likely candidate. Immediately adjacent is mycB encoding a potential DAase that has sequence homology (36% identity) to CghA, which has been implicated to be involved in the cycloaddition during Sch210972 biosynthesis. A trans-acting enoylreductase (ER) is encoded in mycC (Figure 4.2A). This three-gene cassette is found widely among sequenced fungi. To test the link between the gene cluster and production of 1 and 2, which are produced by the wild-type strain at yields of 6 and 50 mg/L, respectively (Figure 4.2B(i), the deletion strain ΔmycA was generated through gene replacement. LC–MS analysis showed that deletion of mycA completely abolished the production of 1 and 2 (Figure 4.2B(ii)), confirming the involvement of MycA in their biosynthesis. Comparison of the LC–MS traces also revealed the abolishment of 3 and 4, previously unreported compounds with the same *m/z* [M + H]^+ of 398, which were purified from the wild-type strain in yields of 10 and 16 mg/L (Figure 4.2C).
Figure 4.2. Verification of the myceliothermophin gene cluster. (A) The myc cluster encodes the PKS-NRPS MycA (KS, ketosynthase; AT, acyltransferase; DH, dehydratase; MT, methyltransferase; KR, ketoreductase; ACP, acyl carrier protein; C, condensation; A, adenylation; PCP, peptidyl carrier protein; R, reductase), the in trans ER MycC, and the putative DAase MycB. (B) Product profiles of wild-type and single-gene-knockout strains of *M. thermophila* and of *A. nidulans* transformed with combinations of myc genes. (C) Structures of metabolites isolated. 3, 4, and 5 are assumed to have similar stereochemistries as 1.

NMR structural characterization showed that 3 is an acyclic polyolefinic compound containing a 4-pyrrolin-2-one moiety that is conjugated with the C18 enol via C19 (Figure 4.2C). This is likely the enolized form of the PKS-NRPS/ER product, which contains eight differentially α- and β-functionalized ketide units aminoacylated with leucine and has undergone Knoevenagel condensation (Figure 4.4). To confirm that 3 is synthesized by PKS-NRPS and ER,
we introduced both *mycA* and *mycC* into the heterologous host *Aspergillus nidulans* A1145. Compared with the untransformed host, *A. nidulans* expressing MycA and MycC showed clear accumulation of a new compound identical to 3 in retention time, UV absorbance, and mass (Figure 4.2B(v)). This suggests that the *myc* PKS-NRPS and its ER partner are sufficient to form the pyrrolinone moiety, in contrast to previous work with other R-domain-containing fungal PKS-NRPS systems.\(^\text{19}\)

On the other hand, 4 is the *trans*-decalin cyclized form of 3 and contains the enolized dienyl pyrrolinone ring. Thus, 4 represents the potential intermediate that can be oxidized into both 1 and 2. To examine the role of MycB on the formation of 1, 2, and 4 in *M. thermophila*, we generated the ΔmycB strain. LC–MS analysis of the metabolites showed that all of these cyclized products were abolished, leaving acyclic 3 as the dominant product (Figure 4.2B(iii)). Hence, MycB is directly involved in the cycloaddition to form the decalin ring. This was further verified when the entire three-gene cassette *mycABC* was introduced into *A. nidulans* (Figure 4.2B(iv)). Analysis of the extract showed that compared with *mycAC* alone, introduction of *mycB* led to the emergence of the cyclized product 4 as well as the final metabolite 1. We could not detect 2 in this strain, suggesting that the stereoselective hydroxylation of C21 is catalyzed by an additional enzyme present only in *M. thermophila*. Analysis of the genes near the *myc* cluster did not reveal any oxygenases that may perform this reaction, and hence, the enzyme may be shared with other pathways in the strain.\(^\text{20}\)

In comparing the various extracts analyzed in Figure 4.2B, we noted a new compound, 5, that is consistently found in *M. thermophila* ΔmycB strain and the transformed *A. nidulans* strains. This compound has *m/z* [M + H]\(^+\) of 396 and \(\lambda_{\text{max}} = 327\) nm, both of which closely resemble those of 1. Purification of 5 from the ΔmycB strain (3 mg/L) followed by NMR
characterization revealed that the new compound is the acyclic form of 1, which is consistent with its accumulation in the ΔmycB and A. nidulans mycAC strains. We propose that 5 could be a product of air oxidation of 3, likely via the same mechanism as for oxidation of 4 to give 1. We attribute the accumulation of 3 and 5 in the A. nidulans mycABC strain to insufficient activity of MycB and/or differences in the redox environment compared with the original producer. Indeed, expressing a second copy of the mycB gene led to a decreased amount of 5 and increased amounts of 4 and 1.

To confirm that MycB is indeed a DAase, N-FLAG-tagged recombinant enzyme was heterologously expressed and purified from Saccharomyces cerevisiae BJ5464-NpgA (0.2 mg/L). Either 0.2 mM 3 or 5 was then incubated with 1 µM MycB in Tris buffer (pH 7.4), and the formation of cyclized products was assayed by LC–MS (Figure 4.3A). No conversion of 3 to 4 was detected under prolonged incubation (3 degraded after overnight assay), while complete conversion of 5 to 1 was observed in 3 h (Figure 4.3A,B). Control incubation in the absence of MycB led to degradation of 5. All of the chromatographic and spectroscopic properties of 1 from the in vitro assay matched those of 1 purified from the in vivo reaction (Figure 4.2B), showing that MycB can catalyze the IMDA reaction with regio- and stereocontrol. Kinetic measurements showed MycB to have $K_M \approx 75$ µM toward 5 and $k_{cat} \approx 0.9$ s$^{-1}$ (Figure 4.3C).
Figure 4.3. Activity of the DAase MycB. (A) MycB can catalyze the cycloaddition of ketone 5 to give 1 but is not reactive toward enol 3. (B) Time course analysis of the conversion of 5 to 1. The starting concentrations of 5 and MycB were 200 and 1 µM, respectively. (C) Saturation kinetics of MycB. Each data point was obtained in triplicate.

The in vitro results confirmed that MycB can indeed catalyze the DA reaction to convert C18 keto 5 into the cytotoxic natural product 1. The failure of MycB to catalyze the conversion of C18 enol 3 into 4, however, suggests differential reactivity toward the ketone and enol substrates. This difference also leads to a proposed myc pathway to account for the isolation of metabolites 1–5 (Figure 4.4). Following MycA-catalyzed construction and release of aminoacyl polyketide aldehyde 6, Knoevenagel condensation yields the expected ketone 7. We propose that
this ketone is most likely the natural substrate of MycB, leading to the formation of the endo product 8. The keto-3-pyrrolin-2-one can readily enolize to form the observed 4. When MycB is inactivated, acyclic 7 can also readily enolize to form 3, which does not undergo the MycB-catalyzed IMDA reaction. However, both 3 and 4 may undergo spontaneous air oxidation to yield 5 and 1, respectively. Indeed, when 4 was dissolved in methanol at 37 °C for 16 h, formation of 1 was observed along with equimolar release of H_2O_2. We proposed an oxidation mechanism that involves sequential single-electron reduction of O_2, during which the C18 enol hydrogen is first abstracted. Significantly (and serendipitously), formation of 5 prevents further tautomerization of the 3-pyrrolin-2-one moiety, resulting in a stable acyclic C18 keto substrate for MycB.
Figure 4.4. Proposed biosynthetic pathway of 1 based on isolated natural products and biochemical characterization of MycB. DFT analyses were performed on model substrates (1′–8′) in which the isobutyl group in 1–8 is replaced by a methyl group. Calculations were performed at the CPCM(water)/M06-2X/6-311+G(d,p)/M06-2X/6-31G(d) level of theory. The computed free energies of model compounds are shown in parentheses. Calculations of stereoselectivity under uncatalyzed and catalyzed conditions are shown in the dashed box.

To support the proposed pathway and rationalize the reactivity difference between the C18 keto and enol substrates in the IMDA reaction, we performed density functional theory (DFT) studies on model substrates and products 1′–8′, in which the isobutyl group in 1–8 is replaced by a methyl group. The enols 3′ and 4′ were found to be more stable than the
corresponding ketones $7'$ and $8'$, respectively, with $\Delta G = -8.0$ and $-3.5$ kcal/mol, respectively (Figure 4.4), supporting the rapid enolization of $7$ into $3$ in the absence of MycB.

We then calculated the energetics of the three potential DA reactions shown in Figure 4.4, starting with either $7'$, $3'$, or $5'$. In each case, the uncatalyzed DA reaction is very slow, with $\Delta G_{\text{uncat}} \approx 24–25$ kcal/mol, corresponding to a rate constant of $-10^{-5}$ s$^{-1}$ at room temperature according to transition state theory. In contrast, the spontaneous DA reaction in the biosynthesis of Sch210972 or equisetin is very fast.$^{12}$ The DA reaction of the enol precursor of Sch210972 has a barrier of only 12 kcal/mol because of the additional electron-withdrawing keto group in the tetramate moiety.$^{12}$ Interestingly, with $7'$, the uncatalyzed reaction is predicted to produce a mixture of diastereomers (Figure 4.4, dashed box), with the unobserved cis-fused decalin $9'$ being the major isomer, resulting from the lower-energy transition state $\text{TS-}7'-\text{exo}$. We propose that a suitable acidic residue in the active site of MycB may accelerate the reaction by lowering the LUMO of the dienophile and stabilizing the transition state through hydrogen bonding to the C18 carbonyl group (in $7$ and $5$ but not $3$) next to the dienophile. Such a catalytic role for a hydrogen-bond-donating residue is also proposed on the basis of the structures of SpnF and PyrI$^{8}$. Additionally, because $\text{TS-}7'-\text{endo}$ is more asynchronous than $\text{TS7'-exo}$, we envisioned that such a catalyst would stabilize the more polarized endo transition state over the exo transition state.

To test our hypothesis, we used $p$-cresol as a model to probe effects of possible catalytic residues such as a tyrosine in the active site of the enzyme. The catalyzed DA reaction of either $7'$ or $5'$ is much faster, with $\Delta G_{\text{cat}} \approx 19$ kcal/mol. This corresponds to a rate constant of $-0.1$ s$^{-1}$ at room temperature, which agrees with the experimentally measured rate ($k_{\text{cat}} \approx 0.9$ s$^{-1}$; Figure 4.3C). Also as predicted, the catalyzed DA reaction of $7'$ is computed to be endo-selective to
give 8', in agreement with the assay results. The H-bond distance in the endo transition state (TS-7'-Y-endo) is shorter than that in the exo transition state (1.74 vs 1.83 Å) because of the more polarized nature of the endo TS. In contrast, the H-bond from cresol to the corresponding enol oxygen in 3' has only a minimal effect on the rate of its DA reaction, with $\Delta G_{\text{cat}*} = 23.9$ kcal/mol. This is in agreement with the experimental observation that MycB could not catalyze the DA reaction of enol 3.

### 4.4 Conclusion

In summary, nanomolar cytotoxic 1 is synthesized by a concise three-enzyme pathway. Our findings expand the collection of sought-after DAases from fungi and may lead to the discovery of new decalin-containing natural products using MycB as a signature biosynthetic marker.
4.5 References


Chapter 5. Distortion-Controlled Reactivity and Molecular Dynamics of Dehydro-Diels-Alder Reactions

5.1 Abstract
We report density functional theory (M06-2X) studies of five dehydro-Diels–Alder (DDA) reactions. For these and the parent reaction, the stepwise mechanisms have similar barriers, whereas the barriers of the concerted mechanisms differ significantly. The reactivity of DDA reactions is controlled by distortion energy. The concerted and stepwise mechanisms of the hexadehydro-Diels–Alder (HDDA) reaction are competitive with activation barriers of ~36 kcal/mol. This is because a large distortion energy (~43 kcal/mol) is required to achieve the concerted transition state geometry. MD simulations reveal that productive concerted trajectories display a strong angle bending oscillation (~25° oscillation amplitude), while the stepwise trajectories show only a chaotic pattern and less pronounced bending vibrations.

5.2 Introduction
The Diels–Alder (DA) reaction between a diene and a dienophile (alkene or alkyne) represents one of the most widely utilized and well-studied synthetic transformations in organic chemistry (Scheme 5.1, eq.1 and eq.2).¹⁻⁵ This (4+2) cycloaddition is particularly useful for constructing 6-membered rings with up to 4 stereogenic centers in a single step, often with good regio- and stereocontrol. Various aspects of the DA reactions, such as mechanism⁶, dynamics⁷, selectivity⁸, asymmetric catalysis⁹, and industrial applications¹⁰, have attracted generations of theoretical and synthetic chemists ever since its original discovery in 1928.¹ Specific types or variants of DA reactions, such as intramolecular (IMDA) and transannular (TADA) reactions¹¹⁻
and hetero-Diels–Alder (HDA) reactions\textsuperscript{15,16}, have been categorized. Novel utilities and new aspects of DA reactions are still emerging. The development of the tetrazine ligation\textsuperscript{17–19} (an inverse electron demand HDA reaction\textsuperscript{20} widely used for bioorthogonal applications) and the search for enzymes that catalyze DA reactions (Diels–Alderases)\textsuperscript{21–23} are recent examples.

In 2008, Wessig et al. described another important variant of the DA reaction, the dehydro-Diels–Alder (DDA) reaction\textsuperscript{24} (Scheme 5.1, eq.3–6). If one or both double bonds in the diene component of a DA reaction are replaced by a triple bond, the product must contain cumulated double bonds (i.e. cyclic allenes).\textsuperscript{25} Due to high strain, these initially formed species usually undergo further reactions such as hydrogen migration or dimerization to generate more stable final products.\textsuperscript{24} The trapping of these reactive intermediates in a controlled fashion was the key to harnessing the synthetic power of DDA reactions. Over the past few years, various research groups have demonstrated the synthetic utility of DDA reactions.\textsuperscript{26–29} One prominent example of these achievements is the development of the intramolecular version of eq.6 (Scheme 5.1), the hexadehydro-Diels–Alder (HDDA) reaction\textsuperscript{30}.

**Scheme 5.1.** Diels–Alder (DA) and dehydro-Diels–Alder (DDA) reactions

\begin{align*}
\text{eq.1} & \quad \text{\text{\large $+$}} \quad \text{\large $\rightarrow$} \quad \text{\large $\textcircled{1}$} \\
\text{eq.2} & \quad \text{\text{\large $+$}} \quad \text{\large $\rightarrow$} \quad \text{\large $\textcircled{2}$} \\
\text{eq.3} & \quad \text{\text{\large $+$}} \quad \text{\large $\rightarrow$} \quad \text{\large $\textcircled{3}$} \\
\text{eq.4} & \quad \text{\text{\large $+$}} \quad \text{\large $\rightarrow$} \quad \text{\large $\textcircled{4}$} \\
\text{eq.5} & \quad \text{\text{\large $+$}} \quad \text{\large $\rightarrow$} \quad \text{\large $\textcircled{5}$} \\
\text{eq.6} & \quad \text{\text{\large $+$}} \quad \text{\large $\rightarrow$} \quad \text{\large $\textcircled{6}$}
\end{align*}
Mechanistically, thermal DA reactions are symmetry allowed pericyclic transformations according to Woodward-Hoffmann rules. In most cases they are concerted processes, although stepwise mechanisms via diradical intermediates operate in some special cases. However, for HDDA reactions, whether or not the reactions are concerted or stepwise has engendered some controversy recently. Schaefer et al. studied the thermal fragmentation of ortho-benzyne (a retro-HDDA reaction), a process that is believed to play an important role in the combustion of aromatic compounds. According to ab initio calculations, the concerted mechanism is consistent with experimental observations. However, it was pointed out “there may also be a competitive, nonconcerted route through an open-chain singlet diradical intermediate, a problem that awaits elucidation”. In 2011, Johnson et al. reported (U)CCSD(T)/M05-2X computational results on the concerted and stepwise pathways for all 6 reactions shown in Scheme 5.1. It was found that a concerted reaction is favored in every case, but the difference between the barriers of the two mechanisms is only 0.5 kcal/mol for the last reaction (eq.6). Recently, Cramer, Hoye and Kuwata reported a combined experimental and computational study of intramolecular HDDA reactions. They found that the stepwise transition states are lower in energy than the corresponding concerted ones, and the computed barriers of reaction are in good agreement with experimentally measured kinetics. While the stepwise mechanism is most likely involved in the intramolecular HDDA reactions of substituted reactants, there is competition between concerted and stepwise mechanisms for the parent reaction (eq.6). Johnson et al. showed that for the series of DA and DDA reactions in Scheme 5.1, the advantage of the concerted mechanism diminishes as the substrate becomes more unsaturated. Our group has studied computationally the mechanism of bimolecular HDDA reactions involving substituted
substrates.\textsuperscript{35} We found that the accelerating effect of alkynyl substituents on the reaction rate is due to the decrease in distortion energy required to achieve the stepwise transition states. Very recently, Hoye and co-workers reported another intriguing type of DDA reactions, the pentadehydro-Diels–Alder (PDDA) reaction.\textsuperscript{36} The PDDA reactions are also likely involving stepwise mechanisms according to density functional theory (DFT) calculations.\textsuperscript{36}

These results prompted us to ask how distortion energies contribute to the increase in concerted barriers for DA and DDA reactions. We have now conducted DFT studies and performed distortion/interaction analyses on the 6 DA and DDA reactions shown in Scheme 5.1. We also performed direct molecular dynamics (MD) simulations on HDDA reactions, to illuminate how reactants distort into transition state geometries in both concerted and stepwise trajectories.

5.3 Computational Methods

All density functional theory (DFT) computations were performed using Gaussian09.\textsuperscript{37} Geometry optimizations were carried out at the (U)M06-2X/6-311+G(d,p) level of theory.\textsuperscript{38} HOMO-LUMO mixing for the initial guess was used for open-shell singlet calculations. Normal vibrational mode analysis at the same level confirmed that optimized structures were minima or transition states (TS). The distortion/interaction analysis is based on “electronic energies (\(E\))”, which are the energies of hypothetically rigid molecules. This is used, instead of \(H\) or \(G\), because the distorted separated fragments are not stationary points, as no harmonic frequency analysis can be performed to obtain \(H\) and \(G\).

(U)M06-2X/6-31G(d) was found to give qualitatively same conclusions about the relative energetics of the reactions studied. Therefore, molecular dynamics (MD) simulations were
performed at the (U)M06-2X/6-31G(d) level of theory. Direct molecular dynamics (MD) simulations were performed for the concerted and stepwise reactions of diyne-yne (Scheme 5.1, eq. 6) in the gas phase. Quasiclassical trajectories (QCTs) were initialized in the region of the potential energy surface near the TS. Normal mode sampling involved adding zero-point energy for each real normal mode in the TS, and performing a Boltzmann sampling of geometries to afford the thermal energy available at 300 K with a random phase. The trajectories were propagated forwards and backwards, 500 fs in each direction. The classical equations of motion were integrated with a velocity-Verlet algorithm using Singleton’s program Progdyn, with the energies and derivatives computed on the fly by the UM06-2X method using Gaussian 09. The step length for integration was 1 fs.

5.4 Results and Discussion

The top row of Figure 5.1 shows the 6 transition structures TS-1–6c for the concerted reactions. For the stepwise reactions, the transition structures TS-1–6s for the formation of diradical intermediates are shown in the bottom row of Figure 5.1. The enthalpies and free energies of activation for the concerted and stepwise reactions are shown below the corresponding TS. The concerted mechanisms are energetically favored over the stepwise mechanisms for eq.1–4. In contrast, TS-5s and TS-6s are lower in energy than TS-5c and TS-6c, respectively. The detailed energetics for all 6 reactions are summarized in Table 5.1 and Table 5.2. These are electronic energies, including no ZPE or thermal corrections, so that the distortion energies can be evaluated. The activation energies and reaction energies agree qualitatively with Johnson’s results. For the concerted pathways (Table 5.1), the barriers of reaction ($E_{act}$) range from 19.6 to 36.0 kcal/mol for the 6 reactions. In contrast, for the stepwise pathways (Table 5.2),
transition states **TS-1–6s** are very close in energy relative to the corresponding reactants (34.2 – 35.4 kcal/mol). The first steps are rate-determining except for the reaction between diyne and ene (entry 5), where the second TS (37.8 kcal/mol) is 3.6 kcal/mol higher in energy than the first TS (34.2 kcal/mol). This might be due to the instability of the product of ring closure, the cyclic cumulene ($E_{\text{rxn}} = -4.4$ kcal/mol, Table 5.1, entry 5). In fact, the diradical would prefer to form a cyclobutene (in a [2+2] cycloaddition) instead of cyclic cumulene. Comparing the $E_{\text{act}}$ values in Table 5.1 to those of Table 5.2 for all 6 reactions, it is clear that the preference for concerted mechanism diminishes as reaction goes from DA (eq.1) to HDDA (eq.6). For the HDDA reaction, **TS-6s** is 0.8 kcal/mol lower in energy than that of **TS-6c**.

**Figure 5.1.** (U)M06-2X/6-311+G(d,p)-optimized transition structures for the concerted and stepwise DA and DDA reactions. Enthalpies and free energies of activation are in kcal/mol.

Both concerted and stepwise transition states (**TS-1–6c** and **TS-1–6s**) were analyzed using the distortion/interaction model,\textsuperscript{40} also known as the activation strain model.\textsuperscript{41} For each reaction, the transition structure is separated into two fragments (diene/enyne/diyne and ene/ynne), followed by single-point energy calculations on each fragment. The difference in energy between
the distorted fragments and optimized ground-state geometries is the distortion energy of diene/enyne/diyne ($E_{\text{dist-4}\pi}$) and ene/yne ($E_{\text{dist-2}\pi}$), respectively. The difference between the activation energy ($E_{\text{act}}$) and the total distortion energy ($E_{\text{dist}} = E_{\text{dist-4}\pi} + E_{\text{dist-2}\pi}$) is the interaction energy ($E_{\text{int}}$). The results are also shown in Tables 5.1 and 5.2.

**Table 5.1.** Activation, distortion, interaction, and reaction energies (in kcal/mol) calculated with M06-2X/6-311+G(d,p) for concerted reactions.

<table>
<thead>
<tr>
<th>entry</th>
<th>TS</th>
<th>$E_{\text{act}}$</th>
<th>$E_{\text{dist-4}\pi}$</th>
<th>$E_{\text{dist-2}\pi}$</th>
<th>$E_{\text{dist}}$</th>
<th>$E_{\text{int}}$</th>
<th>$E_{\text{rxn}}$</th>
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Table 5.2. Activation, distortion, and interaction energies (in kcal/mol) calculated with (U)M06-2X/6-311+G(d,p) for stepwise reactions. Energies of diradical intermediates (DR) and second transition state (TSs2) are also shown.

<table>
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<th>entry</th>
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<th>$E_{\text{dist}}$</th>
<th>$E_{\text{int}}$</th>
<th>$E_{\text{(DR)}}$</th>
<th>$E_{\text{(TSs2)}}$</th>
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For concerted reactions (Table 5.1), distortion energies are the main contributors (26–44 kcal/mol) to the activation barriers, while the favorable (negative) interaction energies are relatively small (-6 to -10 kcal/mol). For each reaction, the distortion energy of the $4\pi$ component ($E_{\text{dist-4}\pi}$) is the main contributor to the total distortion energy, because significant bending distorts the planar or linear molecule into the concerted transition state geometry, where both the termini of the diene overlap with the termini of the dienophile. In contrast, for the stepwise reactions (Table 5.2), the $E_{\text{dist-4}\pi}$ and $E_{\text{dist-2}\pi}$ are similar in magnitude, and approximately constant at 11–16 kcal/mol each. In the stepwise transition states, the $4\pi$ components distort much less than in the concerted transition states. Nevertheless, the total distortion energies are still the controlling factors of activation barriers, whereas the unfavorable (positive) interaction energies are relatively small as well (8–10 kcal/mol). We previously demonstrated that alkynyl substituents accelerate the HDDA reaction by ~5 orders of magnitude, mainly by decreasing the
distortion energy required to achieve the diradical transition state.\textsuperscript{35} In the present study, the distortion energy still predominantly influences the reactivity of unsubstituted DA and DDA reactions. Most notably, the great difference in activation energies ($E_{\text{act}}$) for the 6 concerted reactions is associated with the dramatic change in distortion energies ($E_{\text{dist}}$). Generally, reaction with a higher $E_{\text{dist}}$ tends to have a higher $E_{\text{act}}$, except for the reaction between diyne and ene (Table 5.1, entry 5), in which the interaction energy ($-10.4$ kcal/mol) partially offsets the highest distortion energy (44.3 kcal/mol). This trend is illustrated in Figure 5.2, which is a plot of $E_{\text{act}}$ versus $E_{\text{dist}}$ for the 6 concerted reactions. One manifestation of this trend is that, for the HDDA reaction, the stepwise mechanism becomes competitive, whereas for DA reactions, the concerted mechanisms are energetically favored. The high barrier of the concerted mechanism for the HDDA reaction is attributed to the high distortion energy required to distort the reactants into transition state geometries.
Figure 5.2. Plot of activation energies versus distortion energies for 6 concerted reactions. Energies are in kcal/mol.

We performed direct molecular dynamics (MD) simulations for the concerted and stepwise mechanisms of the diyne-yne (HDDA) reaction, to study how reactants distort into transition state geometries in a time-resolved fashion. In particular, the vibrations that must be excited for reaction to occur are of special interest. Figure 5.3 shows snapshots of two typical reactive trajectories in butadiyne-acetylene cycloadditions, a prototype of HDDA reactions. Figure 5.3a and Figure 5.3b are two trajectories, one for a concerted, and the other for a stepwise pathway, respectively. The bond angles and time at which each snapshot is taken are shown for eight snapshots of each trajectory. As shown in Figure 5.3a, a strong bending motion is observed for butadiyne before it reaches the transition state. The bending angle of butadiyne is 151° at -
250 fs, 177° at -200 fs, 154° at -100 fs, 175° at -50 fs, and 143° at 0 fs. This vibration displays a large bending angle oscillation (~25° oscillation amplitude). The first C-C bond of benzyne forms at 30 fs. We consider bond formation to occur when the first C-C distance reaches 1.6 Å. The time gap between formation of the two bonds is 3 fs. This is a highly dynamically concerted trajectory, since we have defined a dynamically concerted trajectory as one with a time gap of 60 fs or less.\textsuperscript{7,42} In comparison, the period of vibration for a fully formed C-C bond is 30 fs.\textsuperscript{42}

Figure 5.3b shows a stepwise trajectory. From -250 fs to 0 fs, the bending angle ranges from 156° to 172° (~16°). The oscillation pattern of the stepwise trajectory is not as severe as the concerted one. Notably, butadiyne and acetylene mutually rotate as they approach the transition state. The first bond forms at 20 fs, which is comparable to the timing of first bond formation in the concerted pathway. The second bond does not form during the full 500 fs simulation, indicating the formation of a relatively long-lived diradical intermediate.
Figure 5.3. Snapshots for two typical reactive trajectories of butadiyne-acetylene cycloadditions in (a) concerted pathway where butadiyne and acetylene lead to benzyne, and in (b) stepwise pathway in which butadiyne and acetylene gives a diradical intermediate after one C-C bond formation. The intermediate does not give the benzyne intermediate within 500 fs. 0 fs corresponds to the transition state geometry where trajectories are initiated.

Results for 300 butadiyne-acetylene cycloaddition molecular dynamics trajectories are summarized in Figure 5.4. Figure 5.4a shows the 150 concerted and 150 stepwise trajectories, plotted with respect to two forming bond lengths, Bond 1 and Bond 2 labeled on the graph. Trajectories are initiated from the transition state (blue dots shown on the graph). The 98%
confidence interval of the forming bond lengths in the transition state was defined as transition zone. The transition zones in the concerted trajectories are 2.24±0.29 Å for both bonds, and these in the stepwise trajectories are 5.54±0.32 Å for Bond 1, and 1.80±0.05 Å for Bond 2. The concerted and stepwise trajectories largely overlap on the upper right corner of the graph, (4-6 Å for both bonds) which is the region of separated reactants. The trajectories on the concerted pathway move diagonally on the two dimensional PES, because both bond lengths must decrease together to pass the concerted reaction barrier. The stepwise trajectories move vertically, because only Bond 2 forms in crossing the first barrier. Bond 2 is about 1.5 Å in the intermediates while Bond 1 is 4-6 Å, indicating the random rotation of the newly formed Bond 2. During the time of passage beyond TS-6s, only intermediates are formed and none of the diradicals traverse the second transition state region marked by a green oval in Figure 5.4a. The intermediate is 5 kcal/mol below the second TS (Table 5.2, entry 6), and both rotation about Bond 2 and vibrational excitation is required before the second bond can form. This takes picoseconds, not femtoseconds.
Figure 5.4. (a) Distribution of 300 reactive trajectories, 150 for the concerted pathway, and 150 for the stepwise pathway. Blue dots are starting points from normal mode sampling used to
initiate trajectories. Contour plots were calculated with UM06-2X/6-31G(d). Energies are in kcal/mol relative to separated reactants. The Energy scale is shown on the right. (b) The averaged trajectory generated from 150 quasiclassical trajectories represented by bending angle versus time in the concerted butadiyne-acetylene reactions. (c) The averaged trajectory generated from 150 quasiclassical trajectories represented by bending angle versus time in the stepwise butadiyne-acetylene reactions. Bending angles are labeled in red on each TS structure.

Figure 5.4b shows a single trajectory generated from averaging 150 trajectories initiated from the concerted butadiyne-acetylene cycloaddition TS. The averaged trajectory shows a strong oscillation pattern from -500 fs to -50 fs. A concerted single trajectory was conducted to investigate how activation energy is partitioned into translational, rotational and vibrational motions in reactants.\textsuperscript{43,44} The single trajectory was propagated using UM06-2X/6-31G(d) method with no ZPE or thermal energy and with 0.6 kcal/mol kinetic energy in the direction of the reaction coordinate. The 33.9 kcal/mol barrier plus 0.6 kcal/mol kinetic energy is released in these reactants as 15.9 kcal/mol translational energy, 0 kcal/mol rotational energy, and 18.4 kcal/mol vibrational energy.\textsuperscript{45} This result shows the bending excitation in the HDDA reactions as the linear diyne reactants distort to form the TS geometries. The oscillation pattern was previously reported in fluoroethane decomposition to hydrofluoride and ethene,\textsuperscript{46} 1,2,6-heptatriene rearrangement to 3-methylenehexa-1,5-diene,\textsuperscript{47} and several 1,3-dipolar cycloadditions.\textsuperscript{43} In these papers, the oscillation was attributed to a significant bending vibrational excitation that must occur in productive trajectories, and this is the case here, as well. After -50 fs, the bending angle of the averaged trajectory decreases to about 130° to form benzyne product. The oscillation amplitude after forming the product largely decreases.
In contrast, the averaged trajectory for the stepwise reaction displayed in Figure 5.4c does not show a significant oscillation pattern from -500 fs to 0 fs. Energy partition of the stepwise single trajectory shows that the 32.4 kcal/mol barrier plus 0.6 kcal/mol kinetic energy is partitioned 14.4 kcal/mol to translational energy, 11.2 kcal/mol to rotational energy, and 7.5 kcal/mol vibrational energy in the reactants.\textsuperscript{45} The stepwise mechanism involves considerable rotational excitation, and a moderate degree of vibrational excitation during the formation of the first C-C bond. A diradical intermediate is formed after the first bond formation, and the intermediate still survives after 500 fs. The chaotic pattern after 0 fs is suggestive of intramolecular vibrational energy relaxation.

5.5 Conclusion

We report details of the concerted and stepwise mechanisms of six Diels–Alder (DA) and dehydro-Diels–Alder (DDA) reactions. While the stepwise mechanisms have similar barriers for the six prototype reactions studied, the energies of concerted transition states differ significantly in the series. Distortion/interaction analyses reveal that this difference originates from the distortion energy required to achieve the transition states. One manifestation of this distortion-controlled reactivity of DDA reactions is that the stepwise HDDA reaction is competitive with the concerted mechanism.

Direct molecular dynamics (MD) simulations for the concerted and stepwise mechanisms of the HDDA reaction were conducted. From -500 fs to 0 fs, the concerted trajectories display a strong bending angle oscillation (~25° oscillation amplitude), while the stepwise trajectories show a relatively chaotic pattern, and rotational motion instead. The concerted mechanism of the HDDA reaction involves high bending vibrational excitation, because linear diyne reactants need
to be highly bent to form the TS geometries. In contrast, the stepwise mechanism requires less geometrical deformation to enable the first C-C bond formation.

The distortion/interaction analyses, based upon the transition structures computed for each reaction reflect the observations from MD trajectory simulations. That is the more highly distorted the transition state, the greater the necessity for excitation of the corresponding vibrational modes in order to achieve productive trajectories.
5.6 References

(1) Diels, O.; Alder, K. Justus Liebigs Annalen der Chemie 1928, 460, 98.


The distortion/interaction model has been used to study the mechanism and reactivity of a variety of cycloaddition reactions. For relevant studies, see: (c) Liu, S.; Lei, Y.; Qi, X.; Lan, Y. J. Phys. Chem. A 2014, 118, 2638. (d) Qi, X.; Li, Y.; Zhang, G.; Li, Y.; Lei, A.; Liu, C.; Lan, Y. Dalon Trans. 2015, 44, 11165.

The energy partition of single trajectory is detailed in the supporting information. The partition energies in reactants are averaged over 1000 fs in single trajectory after the reactants are separated by 5 Å. The error in average partition energy is about 0.2 kcal/mol, which causes the slight difference between the energy barrier and the sum of partition energies in the reactants.
Chapter 6. Mechanisms and Origins of Selectivities of the Lewis Acid-Catalyzed Diels–Alder Reactions between Aryl Allenes with Acrylates

6.1 Abstract

The mechanisms of recently reported Lewis acid-catalyzed Diels–Alder reactions of aryl allenes and acrylates were studied using density functional theory calculations. A stepwise mechanism involving short-lived zwitterion intermediates is established. The reaction is endo-selective in the presence of Lewis acid catalyst. The [2+2] cycloaddition is not observed because of the greater charge separation in the first step of the [2+2] cycloaddition. The origins of chirality transfer in the Diels–Alder reaction using chiral aryl allenes are uncovered, and the absolute stereochemistry of the product is predicted.

6.2 Introduction

The Diels–Alder (DA) reaction of dienes and alkenes is one of the most useful ring-forming reactions in organic chemistry.\textsuperscript{1–5} Despite numerous advances since it was established in 1928, the scope of this powerful transformation has been continuously expanded.\textsuperscript{6–11} Recently, in their quest to develop [2+2] cycloaddition reactions to synthesize cyclobutanes, Conner and Brown discovered an unexpected Lewis acid-catalyzed DA reaction of aryl allenes and acrylates, involving the aromatic ring as part of the diene (Scheme 6.1).\textsuperscript{12}
Based on their previous work, the authors were expecting a [2+2] cycloaddition of aryl allene and acrylate to produce a cyclobutane (Scheme 6.1a). However, the observed product of the reaction between 1 and 2 is dihydronaphthalene 4 (Scheme 6.1b).

The observed reaction was proposed to proceed via a concerted asynchronous [4+2] transition state to form a de-aromatized intermediate, followed by a formal 1,5-H shift to afford the final product. The reaction is also suggested to follow the “endo rule”. However, the key intermediate could not be observed, and this stereochemistry could not be confirmed. We have undertaken a density functional theory (DFT) study to investigate the mechanisms and origins of product selectivity of this unusual Diels–Alder reaction. We have also explored the [2+2] cycloaddition reaction, to explain why it was not observed.
6.3 Computational Methods

All density functional theory (DFT) calculations were performed using Gaussian 09.\textsuperscript{15} Geometry optimizations and frequency calculations were performed at the M06-2X/6-31G(d) level of theory.\textsuperscript{16} Solvent effects were modeled with the conductor-like polarizable continuum model (CPCM) using dichloromethane as the solvent.\textsuperscript{17} Normal vibrational mode analysis confirmed that optimized structures are minima or transition structures. Truhlar’s quasiharmonic correction was used to compute molecular entropies to reduce error caused by the breakdown of the harmonic approximation, by setting all positive frequencies that are less than 100 cm\textsuperscript{−1} to 100 cm\textsuperscript{−1}.\textsuperscript{18} CPCM(dichloromethane)-M06-2X/6-311+G(d,p) single-point energies were computed on the CPCM(dichloromethane)-M06-2X/6-31G(d)-optimized structures. All 3D renderings of stationary points were generated using CYLview.\textsuperscript{19} GaussView\textsuperscript{20} and Avogadro\textsuperscript{21} were used to construct initial structures used in our computations.

6.4 Results and Discussion

6.4.1 Uncatalyzed Diels–Alder reaction

We first studied the uncatalyzed Diels–Alder reaction between phenyl allene \textbf{1} and methyl acrylate \textbf{2}. Figure 6.1 shows the optimized transition structures (TS) and free energy profiles for the \textit{endo} and \textit{exo} pathways of the reaction. Both \textbf{TS-endo} and \textbf{TS-exo} are concerted yet highly asynchronous, each with a shorter forming bond at \(\sim\)1.9 Å and a longer forming bond at \(\sim\)2.6 Å. \textbf{TS-endo} is slightly favored over \textbf{TS-exo} (\(\Delta\Delta G^\ddagger = 1.0\) kcal/mol) although neither process is likely to proceed at room temperature due to the high barriers of reaction (\(\sim\)34 kcal/mol). The Diels–Alder adducts are about 7 kcal/mol more stable than the reactants. The
formal [1,5] H shift process is highly exergonic because of re-aromatization and the formation of more substituted alkene in the final product.

**Figure 6.1.** Transition structures and free energy profile for the uncatalyzed Diels–Alder reaction between 1 and 2.

### 6.4.2 AlCl₃-catalyzed Diels–Alder reaction

The association of AlCl₃ with the carbonyl oxygen of 2 is highly exergonic with a computed ΔG of −25.0 kcal/mol. The association of AlCl₃ with the final product 4 is even stronger (−26.2 kcal/mol). This rationalizes the need for a relatively high amount of catalyst loading (30 mol%), since product inhibition will occur. Figure 6.2 shows the optimized *endo* and *exo* transition structures, intermediates and the computed free energy profiles for the DA reactions in the presence of AlCl₃.
Figure 6.2. Transition structures, intermediates and free energy profile for the Diels–Alder reaction between 1 and 2-L.

The Diels–Alder reaction is much faster with the carbonyl oxygen of the dienophile coordinated to AlCl₃ (2-L). The free energy barrier is now only 19.8 kcal/mol through the endo transition state (TS-1-endo). This corresponds to an enormous (~10¹⁰ fold) rate enhancement compared to the uncatalyzed reaction. The transition structures are also earlier in terms of forming bond lengths. There is essentially only one forming bond at ~2.0 Å, and a stable zwitterion intermediate could be located on the potential energy surface for both the endo and the exo pathways (INT-N and INT-X). However, the intermediates are predicted to be exceedingly short-lived, with the second step to form the DA-adduct being almost barrierless (ΔG‡ = ~1.0 kcal/mol). This is in agreement with the experimentally observed stereospecificity of the reaction...
using deuterium-labeled acrylate. **TS-1-exo** is 2.5 kcal/mol higher in energy than **TS-1-endo**. The catalyzed reaction is not only faster than the uncatalyzed one, but also more *endo*-selective. The origins of the stereoselectivity will be discussed in section 6.4.4.

6.4.3 AlCl₃-catalyzed [2+2] cycloaddition

We next investigated the mechanisms of the [2+2] cycloaddition of 1 and 2-L. The reaction was found to be stepwise as well. Different approaching geometries and conformations were explored. For the formation of the first bond, 13 transition structures that are within 2 kcal/mol relative to the lowest energy TS were located. The lowest energy reaction pathway is shown in Figure 6.3.
The [2+2] cycloaddition of 1 and 2-L has a free energy barrier of 24.9 kcal/mol via TS-3. The forming bond length is ~2 Å, similar to that of the first TS of the Diels–Alder reaction. A stable zwitterion intermediate (INT) can also be obtained. However, the second step has a substantial barrier of ~7 kcal/mol via TS-4 to form the cyclobutane. The first step is rate determining. It is 5 kcal/mol higher in energy than the first step of the DA reaction, thus is predicted not to compete with the observed Diels–Alder reaction.

6.4.4 Origins of the preference of Diels–Alder over [2+2] cycloadditions
To probe the origins of the *endo*-selectivity of the DA reaction and to explain why the [2+2] TS has a much higher barrier than the DA reaction, we analyzed the transition structures using the distortion/interaction model, also known as the activation strain model. Each transition structure is separated into two fragments, followed by single-point energy calculations on each fragment. The difference in energy between the distorted fragments and optimized ground-state geometries is the distortion energy ($E_{\text{dist}}^{\dagger}$), or the activation strain. The difference between the electronic energy of activation ($\Delta E^{\dagger}$) and the distortion energy is the interaction energy ($E_{\text{int}}^{\dagger}$).

We have also computed the dipole moments and plotted the electrostatic potential (ESP) maps of the transition structures. The results are shown in Figure 6.4.

![Figure 6.4](image)

**Figure 6.4.** Electrostatic potential (ESP) maps, dipole moments, and distortion/interaction analysis of TS-1-endo, TS-1-exo and TS-3.

The free energies ($\Delta G^{\dagger}$) of TS-1-exo and TS-3 are higher than that of TS-1-endo by 2.5 and 5.1 kcal/mol, respectively. The corresponding electronic energies of activation ($\Delta E^{\dagger}$) follow
the same trend. The distortion energies are essentially identical for the three transition structures (at ~24 kcal/mol). This results from the great asynchronicity in these three structures, all of which have forming bond length of 1.98–2.02 Å. **TS-1-endo** has the most favorable interaction energy of –20.2 kcal/mol, followed by **TS-1-exo** (–17.9 kcal/mol), while **TS-3** has the least favorable interaction energy (–14.8 kcal/mol). The difference in interaction energy is due to different electrostatic interactions between the two fragments in the TS. The electronegative region (red in the ESP) in **TS-1-endo** is right below the electropositive (blue) phenyl ring, while **TS-1-exo** and **TS-3** have more separation of charges. The computed dipole moments of the three TS also support this hypothesis, as they correlate with the relative energies.

### 6.4.5 Chirality transfer and the origins of stereoselectivity

Modest transfer of chirality was observed when chiral aryl allene was used (Scheme 6.2), although the absolute stereochemistry of the products is unknown.

**Scheme 6.2.** Diels–Alder reaction of chiral allene (R)-6 and methyl acrylate 2

![Scheme 6.2. Diels–Alder reaction of chiral allene (R)-6 and methyl acrylate 2](image)

We have modeled this process and predicted (S)-7 to be the major product. The results are shown in Figure 6.5.
Figure 6.5. Lowest-energy transition structures and the corresponding products for the Diels–Alder reaction of chiral allene \((R)-6\).

The four transition structures are all endo, with \textbf{TS-(S)-1} and \textbf{TS-(S)-2} (different conformations of acrylate) leading to \((S)-7\), and \textbf{TS-(R)-1} and \textbf{TS-(R)-2} leading to \((R)-7\). The transition structures leading to \((R)-7\) are disfavored due to the steric clashes between the methyl hydrogen of the allene and the alkene hydrogen of the acrylate (2.04 and 2.01 Å as shown in Figure 6.5). This unfavorable interaction is absent in the transition structures leading to \((S)-7\). The computed \(\Delta \Delta G^\ddagger\) agree well with the experimentally measured enantiomeric ratio of the two products, and \((S)-7\) is predicted to be the major one.
6.5 Conclusion

The Lewis acid-catalyzed Diels–Alder reactions of aryl allenes and acrylates involve stepwise mechanisms in which short-lived zwitterion intermediates are formed. The reaction is endo-selective in the presence of Lewis acid catalyst. The [2+2] cycloaddition is not observed because of the greater charge separation in the first step of the [2+2] cycloaddition. We have predicted the absolute stereochemistry of the product of the Diels–Alder reaction using chiral aryl allenes, and identified the origins of the observed stereoselectivity.
6.6 References


Chapter 7. Origins of Regioselectivity in 1,3-Dipolar Cycloadditions of Nitrile Oxides with Alkynylboronates

7.1. Abstract
Density functional theory (M06-2X) studies of the regioselectivity of 1,3-dipolar cycloaddition reactions of benzo and mesitonitrile oxides with alkynyl pinacol and MIDA boronates are reported. Calculated relative free energies of activation reproduce the experimentally observed product ratios. The electronic energies of activation are found to be mainly controlled by distortion energies required to achieve the transition states. Both electronic and steric effects influence regioselectivities.

7.2 Introduction
Isoxazoles are found in a number of drugs such as valdecoxib, a non-steroidal anti-inflammatory agent (Figure 7.1). The 1,3-dipolar cycloaddition reaction is one of the most useful reactions for the synthesis of isoxazoles.\textsuperscript{1,2} Organoboron reagents have been utilized in the 1,3-dipolar cycloaddition reactions of nitrile oxides and alkynylboronates to form 3,4- and 3,5-substituted isoxazole boronic esters (Figure 7.2), as reported by Harrity and co-workers.\textsuperscript{3} The isoxazole boronic esters can be further transformed by Pd-catalyzed cross-coupling reactions. This methodology is promising for the synthesis of a variety of isoxazoles.\textsuperscript{3,4}
**Figure 7.1.** Valdecoxib, a non-steroidal anti-inflammatory drug (NSAID) that contains an isoxazole unit.

![Chemical Structure](image)

**Figure 7.2.** 1,3-Dipolar cycloaddition reaction of nitrile oxide and alkynyl boronic acid pinacol ester.

Recently, Hamann and co-workers reported the synthesis of 3,5-substituted isoxazole boronic esters by 1,3-dipolar cycloadditions of ethynyl boronic acid MIDA (N-methyliminodiacetic acid) esters\(^5\) and nitrile oxides (Figure 7.3, reaction (1)).\(^6\) The reaction between ethynyl boronic acid MIDA ester and benzonitrile oxide only yields the 3,5-substituted product as a single regioisomer, in contrast to the results with the corresponding pinacol esters (Figure 7.3, reactions (2) and (3)).\(^3,6\) Hamann and coworkers hypothesized that the complete regioselectivity in the case of the MIDA ester is due to the presence of the sp\(^3\)-hybridized boron center, which is more sterically demanding in the case of the MIDA boronates compared to the sp\(^2\)-hybridized boron of the pinacol esters.\(^6\) Nitrile oxides in these reactions were generated in situ from the corresponding hydroximic acid chlorides.\(^3,6\)
Figure 7.3. 1,3-Dipolar cycloaddition reactions of nitrile oxides (generated in situ) with ethynyl boronic acid esters.\textsuperscript{3,6}

While the pinacol boryl group should be electron-withdrawing due to the partially vacant boron orbital, it is not so clear whether the MIDA boryl is electron-withdrawing or electron-donating.

In reaction (3), the bulkier and electron-rich mesityl group is present on the nitrile oxide. If steric hindrance were governing the regioselectivity of the reaction, then the ratio of 3,5- to 3,4-substituted product in reaction (3) would be larger than the ratio of 3,5- to 3,4-substituted product in reaction (2). However, the ratio of 3,5- to 3,4-substituted product in reaction (3) is much smaller than expected. This unexpectedly lower ratio suggests that steric hindrance is not the only factor governing the regioselectivity of the reaction. We now present detailed computations to explain the regioselectivity of reactions (1–3).
7.3 Computational Methods

Avogadro\(^8\) and Gaussview\(^9\) were used to create initial structures of reactants (nitrile oxides and alkynylboronates) and products (isoxazole boronic esters). All density functional theory calculations were performed using Gaussian09\(^{10}\). Geometry optimizations and frequency calculations of reactants and transition states were performed at the M06-2X level using the 6-31G(d) basis set.\(^{11,12}\) Single point energies were calculated on the optimized structures at the M06-2X level using the 6-311+G(d,p) basis set. Solvation effects in acetonitrile and diethyl ether were computed by a self-consistent reaction field (SCRF) using the SMD solvent model.\(^{13}\) The frontier molecular orbitals (FMOs) and their energies were computed at the HF/6-31G level on the M06-2X/6-31G(d) optimized geometries. CYLview\(^{14}\) was used to create images of optimized structures. Chemcraft\(^{15}\) was used to create images of molecular orbitals.

7.4 Results and Discussion

The transition structures (TS) and activation energies for the three reactions are shown in Figure 7.4. The TS leading to the 3,5-substituted product of a reaction is denoted ‘TS-Xa’, where X is the number of the reaction being discussed. The transition state leading to the 3,4-substituted product of a reaction is denoted ‘TS-Xb’, accordingly. The free energy barriers for the 3 reactions are all about 28 kcal/mol. The difference in activation free energies (\(\Delta\Delta G^\ddagger\)) between the reaction (1) transition states is 5.2 kcal/mol. This difference in activation energies is in good agreement with Hamann’s findings of complete regioselectivity favoring the 3,5-product when the MIDA ester was used.\(^6\) The relative electronic energy (\(\Delta\Delta E^\ddagger\)) of the two transition states is slightly smaller, 4.5 kcal/mol. For reaction (2), involving the pinacol boronic ester, the
calculated $\Delta\Delta G^\dagger$ is 1.2 kcal/mol. The experimental product ratio is 9 to 1, favoring the 3,5-product.\(^3\) This ratio corresponds to a difference in activation energies of 1.3 kcal/mol at room temperature, according to transition state theory. This is in good agreement with the calculated $\Delta\Delta G^\dagger$ value (1.2 kcal/mol). Interestingly, the calculated $\Delta\Delta E^\dagger$ is −0.9 kcal/mol, favoring the minor product. For reaction 3, in which the larger mesityl group is present, the calculated $\Delta\Delta G^\dagger$ further decreases to 0.6 kcal/mol. This is in good agreement with the experimental product ratio of 2.5:1. The $\Delta\Delta E^\dagger$ is −2.2 kcal/mol, favoring the minor product even more, compared to reaction (2).
Figure 7.4. Transition structures for the 3 reactions of nitrile oxides with ethynyl boronic esters.

To gain more insight into the factors controlling the trends in activation energies, the six transition states were analyzed using the distortion/interaction model,\textsuperscript{16,17} also known as the activation strain model.\textsuperscript{18,19} For each reaction, the transition structure is separated into two fragments (dipole and dipolarophile), followed by single-point energy calculations on each
distorted fragment. The difference in energy between the distorted fragments and optimized ground-state geometries is the distortion energy of dipole ($E_{\text{dist}-4\pi}$) and dipolarophile ($E_{\text{dist}-2\pi}$), respectively. The difference between the activation energy ($\Delta E^\ddagger$) and the total distortion energy ($E_{\text{dist}} = E_{\text{dist}-4\pi} + E_{\text{dist}-2\pi}$) is the interaction energy ($E_{\text{int}}$). The results are shown in Figure 7.5. The black arrows correspond to the activation energies ($\Delta E^\ddagger$). The green arrows correspond to the distortion energies of the dipolarophiles ($E_{\text{dist}-2\pi}$). The blue arrows correspond to the distortion energies of the dipoles ($E_{\text{dist}-4\pi}$). The total length of blue and green arrows corresponds to the total distortion energy ($E_{\text{dist}}$). The red arrows correspond to the favorable interaction energies ($E_{\text{int}}$).

**Figure 7.5.** Graph of distortion, interaction, and activation energies for the three reactions discussed previously (green: distortion energy of dipolarophile, blue: distortion energy of dipole, red: interaction energy, black: activation energy).

For each of the six transition states, the $E_{\text{dist}-4\pi}$ is the main contributor to the $E_{\text{dist}}$. This observation is quite general in 1,3-dipolar cycloadditions and Diels–Alder reactions.17,20 The
$E_{\text{dist-2}\pi}$ is relatively small and constant, except for TS-1b. The dipolarophile distortion is 8.6 kcal/mol for TS-1b, whereas the others are 5.2–5.7 kcal/mol. In TS-1b (Figure 7.4), the C–C–B angle is 149°, whereas the same angles in the other 5 TS ranges from 161–174°. This greater deviation from linear structure accounts for the much higher distortion energy of the dipolarophile in TS-1b is likely due to the steric repulsion between the nitrile oxide phenyl group and the bulky MIDA group. Comparing the two TSs in reaction (1), $E_{\text{dist-2}\pi}$ of TS-1b is 3.0 kcal/mol higher than that of TS-1a. The much higher activation energy of TS-1b than that of TS-1a is mainly attributed to the higher distortion energy arising from steric effects. The larger MIDA substituent on the alkyne strongly disfavors the formation of the 3,4-substituted product.

In reaction (2), the −0.9 kcal/mol difference in $\Delta E^\ddagger$ favoring TS-2b mainly originates from the distortion of the dipole ($E_{\text{dist-2}\pi}$). $E_{\text{dist-4}\pi}$ of TS-2b is 1.0 kcal/mol lower than that of TS-2a. In the TS structures, the N–C–C angle of TS-2b is 147° compared to that of 143° in TS-2a. This is an unusual example in which the $\Delta \Delta E^\ddagger$ and $\Delta \Delta G^\ddagger$ have opposite signs. It is likely due to the fact that the more crowded TS, TS-2b, has less vibrational freedom and, therefore, less favorable entropy. In reaction (3), the −2.2 kcal/mol difference in $\Delta E^\ddagger$ favoring TS-3b mainly originates from the distortion of the dipole ($E_{\text{dist-4}\pi}$). $E_{\text{dist-4}\pi}$ of TS-3b is 2.3 kcal/mol lower than that of TS-3a. In the TS structures, both the N–C–C angle (145°) and the O–N–C angle (141°) of TS-3a are smaller (more distorted) compared to those of TS-3b.

Replacing the phenyl group with a mesityl group generally increases the favorable interaction energies (more negative), as found in going from reaction 2 and 3. This is due to the electron-donating nature of methyl groups on the phenyl ring. To explore this observation, we computed the energies of the frontier molecular orbitals (FMO) of the two dipoles and two dipolarophiles. The results are shown in Figure 7.6.
Figure 7.6. Frontier molecular orbitals (FMO) involved in the 1,3-dipolar cycloaddition of nitrile oxides and alkynylboronates. From left to right: the HOMO and LUMO of the benzonitrile oxide generated in situ in reactions (1) and (2) (denoted 1a), the HOMO and LUMO of the mesitonitrile oxide generated in situ in reaction (3) (denoted 8a), the HOMO and LUMO of the pinacol boronic ester used in both reactions (2) and (3), and the HOMO and LUMO+2 of the MIDA boronate used in reaction (1).

In reactions (2) and (3), the HOMOs of the dipoles (1a and 8a, respectively) interact with the LUMO of the dipolarophile (5). The HOMO of 8a is 0.4 eV higher in energy than that of 1a, due to the electron-donating methyl groups present in the latter structure. As a result, the HOMO of 8a interacts more strongly with the LUMO of 5. This is in agreement with the more favorable interaction energy of reaction (3) compared to that of reaction (2). In reaction (1), the
dominating interaction is between the LUMO of 1a and the HOMO of 2. This is an example of inverse-electron-demand 1,3-dipolar cycloadditions.

7.5 Conclusion

The regioselectivities of three 1,3-dipolar cycloadditions of nitrile oxides with alkynylboronates have been elucidated using density functional theory (M06-2X). The calculated free energy barriers of reaction reproduce the experimentally observed product ratios. Distortion/interaction analyses reveal that the regioselectivity is mainly governed by the distortion energies. With ethynyl boronic acid MIDA ester, the reaction does not afford the 3,4-substituted isoxazole product, due to the large distortion energy required to achieve the corresponding transition state. With ethynyl pinacol boronic ester, the reaction produces a mixture of two regioisomeric products. The electronic energies of activation favor the minor products, due to smaller distortion energies required to achieve the minor transition states. When benzonitrile oxide is replaced with mesitonitrile oxide, the favorable interaction energies increase. This can be explained by the electron-donating nature of the methyl groups present on the mesityl group.
7.6 References


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Chapter 8. Diazo Esters as Dienophiles in Intramolecular (4+2) Cycloadditions: Computational Explorations of Mechanism

8.1 Abstract
The first experimental examples of Diels–Alder (DA) reactions of diazo compounds as heterodienophiles with dienes have been studied with density functional theory (DFT) using the M06-2X functional. For comparison, the reactivities of diazo esters as dienophiles or 1,3-dipoles with 1,3-dienes in intermolecular model systems have been analyzed by the distortion/interaction model. The 1,3-dipolar cycloaddition is strongly favored for the intermolecular system. The intramolecular example is unique because the tether strongly favors the (4+2) cycloaddition.

8.2 Introduction
The Diels–Alder (DA) reaction is one of the most important ring-forming reactions in organic synthesis. While DA reactions between dienes and azo compounds are common, reactions of diazo compounds as dienophiles have not been reported until recently. In contrast, the 1,3-dipolar cycloadditions of diazo compounds to alkenes resulting in the formation of pyrazolines (Scheme 8.1, left) were discovered by Curtius and Buchner in the 1880s, and identification of this as one of many 1,3-dipolar cycloaddition reactions was made by Huisgen more than 50 years ago. In the ensuing time, 1,3-dipolar cycloaddition reactions of diazo compounds with alkenes or alkynes have been well developed and subjected to wide utilization for heterocycle syntheses with tautomerization or dinitrogen extrusion being potential subsequent processes. Diazonium ions have been reported as dienophiles, but diazo esters are well-known to react as dipoles in 1,3-dipolar cycloadditions. This stands in contrast to the
cumulenic ketene and allene counterparts, which generally react as dienophiles or dipolarophiles in (4+2) and (2+2) cycloaddition reactions.\textsuperscript{10–12}

**Scheme 8.1.** Potential cycloadditions of dienes with diazo compounds

![Scheme 8.1](image)

Recently, Doyle et al. reported the first (4+2) cycloaddition of a diazoalkane in which the N=N bond acts as the dienophile.\textsuperscript{13} Treatment of propargylphenyldiazoacetates 4 with 5 mol\% AuCl(Me\textsubscript{2}S) in toluene at 20 °C and pyridine-N-oxide resulted in the formation of (Z)-1 and products 2 anticipated from the diene-diazo cycloaddition. The (E)-1-(1,3-dienyl) aryl diazoacetates (E)-1 (depicted in Scheme 8.2) undergo intramolecular (4+2) cycloadditions to form the azomethine imine 1,3-dipole structures 2, in moderate to high yields. Cycloaddition is first order in (E)-1, and both the activation parameters and their Hammett relationships are consistent with those for other intramolecular Diels-Alder reactions.\textsuperscript{14} We have undertaken a theoretical study of this reaction and report the factors that lead to this anomalous reaction.
We have employed quantum mechanical calculations to characterize the reaction mechanism and transition states for cycloadditions between 1,3-dienes and diazo esters. We have also applied the distortion/interaction model and FMO analysis to investigate the analogous intermolecular cycloadditions between 1,3-dienes and diazo esters.

### 8.3 Computational Methods

All the calculations were performed with Gaussian 09.\(^\text{15}\) Geometry optimizations and frequency calculations of reactants, transition states, and products were carried out with the M06-2X density functional\(^\text{16,17}\) with the 6-31G(d) basis set, which has been found to give relatively accurate geometries for cycloadditions.\(^\text{18}\) Single-point energies were computed at the more accurate M06-2X/6-311+G(d,p) level using the M06-2X/6-31G(d) geometries. The FMO energy gaps of diazo ester and 1,3-diene were calculated with the Hartree-Fock (HF)\(^\text{19–21}\) method with 6-31G(d) basis set using the M06-2X/6-31G(d) geometries. Fragment distortion and interaction energies were computed at the M06-2X/6-311+G(d,p) level. Chloroform (\(\varepsilon = 4.71,\)
$R_{\text{solv}} = 2.48$ Å) was used in the SMD$^{22}$ continuum solvation model single-point calculations on gas-phase optimized geometries. All stationary points were verified as minima or first-order saddle points by a vibrational frequency analysis. Optimized structures are illustrated using CYLview.$^{23}$

8.4 Results and Discussion

Experimentally, the (E)-1-(1,3-dienyl) aryldiazoacetates (E)-1 were found to undergo intramolecular (4+2) cycloadditions to form the azomethine imine product 2. The reaction only occurs with the (E)-1 isomer, not the (Z)-1 isomer.

8.4.1 E-isomer intramolecular cycloadditions

Intramolecular (4+2) and (3+2) cycloadditions of diazo esters with (E)-alkenes are depicted in Figure 8.1. The observed product from the (4+2) cycloaddition reaction is formed with a barrier of only 24.1 kcal/mol in chloroform. The (3+2) cycloaddition reaction has a much higher barrier of 39.6 kcal/mol in chloroform. The (3+2) cycloaddition at the other double bond was not computed, because it would generate a six-membered ring that contains a trans double bond. The product 2b is predicted to be less stable compared to the (4+2) cycloaddition product 2a. The dihedral angle highlighted in green of TS-(4+2)-E is 174.0°, close to -177° as in the reactant. The same dihedral angle of TS-(3+2)-E is -104.9°. This dramatically reduces the conjugation between the ester carbonyl and the diazo group. In fact, when this dihedral angle was constrained to -105° in the reactant, the energy increases by ~12 kcal/mol. The dihedral angle of the ester linkage structure of TS-(4+2)-E is 178.6°, close to the stable s-trans conformation (180°). This angle is -164.0° in TS-(3+2)-E and it is less stable than TS-(4+2)-E.
The forming five-membered ring of TS-(4+2)-E is more stable than the forming four-membered ring of TS-(3+2)-E. This result agrees with the exclusive formation of the (4+2) product from the (E)-alkene observed in experiment.

**Figure 8.1.** Relative free energies (ΔG$_{298}$) (in kcal/mol) of intramolecular (4+2) and (3+2) cycloadditions of diazo ester with (E)-alkenes calculated at the M06-2X/6-311+G(d,p)/SMD(chloroform)//M06-2X/6-31G(d) level. Bond lengths are in Å.

### 8.4.2 (E)-alkene versus (Z)-alkene

Figure 8.2 shows the most stable structure of (E)-alkene and (Z)-alkene. The s-cis conformation of (E)-1 is less stable than the s-trans conformation of (Z)-1, because the (E)-1 structure has steric repulsion between the phenyl group and the neighboring cyclopentenyl.
Nevertheless, no products from the (Z)-alkenes are observed. We explored the possible
cycloadditions of the (Z)-alkene to understand their lack of reactivity. Figure 8.3 shows the
energetics and transition states of the intramolecular (4+2) and (3+2) cycloadditions of diazo
esters with (Z)-alkenes. The transition state of the (3+2) cycloaddition in reaction (c) has the
lowest energy of 38.5 kcal/mol in chloroform, much too high to be observed under the reaction
conditions. The dihedral angles ($C^d-C^1-N^2-C^3$) involving the two forming bonds are -0.6°, 7.4° in
reaction (b) and (c), but -49.3° in reaction (a). In a prototypical Diels-Alder transition state, these
forming bonds are approximately coplanar (dihedral angle near 0°), to maximize overlap at both
termini of the diene. This is not possible in reaction (a), and the TS-(4+2)-Z dihedral angle is
very distorted, and the forming bonds are asynchronous.

The dihedral angle of the ester linkage ($C^d-O^5-C^6-O^7$) for TS-(3+2)-Z' in reaction (b) is -173.0°, close to the stable s-trans conformation (180°) of esters in general, but that of TS-(4+2)-Z in
reaction (a) is 137.0°, and in reaction (c) the dihedral angle ($C^5-O^6-C^7-O^8$) is 101.9°, close to
the least stable conformation (90°). Each of these has some unfavorable strains due to the tether, and none occurs under the reaction conditions.

**Figure 8.3.** Relative free energies ($\Delta G_{298}$) (in kcal/mol) of intramolecular (4+2) cycloaddition for (Z)-alkenes and (3+2) cycloaddition across the diazoalkenes 1,3-dipole for different double bonds of (Z)-alkenes calculated at the M06-2X/6-311+G(d,p)/SMD(chloroform)//M06-2X/6-31G(d) level. Bond lengths are in Å. Relevant dihedral angles are shown in the Table 8.1.
Table 8.1. Relevant dihedral angles for the intramolecular (4+2) and (3+2) cycloadditions for (Z)-1.

<table>
<thead>
<tr>
<th>Dihedral Angles (deg)</th>
<th></th>
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<tbody>
<tr>
<td>TSs</td>
<td>C⁴−C¹−N²−C³</td>
</tr>
<tr>
<td>TS-(4 + 2)-Z</td>
<td>−49.3 (C⁴−N¹−N²−C³)</td>
</tr>
<tr>
<td>TS-(3 + 2)-Z¹</td>
<td>−0.6</td>
</tr>
<tr>
<td>TS-(3 + 2)-Z²</td>
<td>7.4</td>
</tr>
</tbody>
</table>

8.4.3 Intermolecular (4+2) versus (3+2) cycloadditions for (E)-1

To assess the intrinsic barriers of the (4+2) and (3+2) cycloadditions, we have computed the intermolecular versions of the two reactions. All possible (3+2) and (4+2) cycloaddition reactions between the truncated reactants were considered. Figure 8.4 shows the two most stable transition structures for each reaction. The free energy of activation for the (4+2) cycloaddition in chloroform is 39.3 kcal/mol, and the (3+2) cycloaddition has a barrier of 33.4 kcal/mol. The free energy barrier of the (4+2) cycloaddition is now 6 kcal/mol higher than (3+2) cycloaddition. That is, the (3+2) cycloaddition is strongly favored for the intermolecular reaction, when there are no constraints from the tether. Both of these bimolecular reactions have unfavorable entropies, and their $-TΔS^\ddagger$ are −12 kcal/mol.
Figure 8.4. Transition structures and free energies of activation ($\Delta G_{298}$) (in kcal/mol) for the intermolecular (4+2) and (3+2) cycloaddition reactions of truncated reactants calculated at the M06-2X/6-311+G(d,p)/SMD(chloroform)//M06-2X/6-31G(d) level. Bond lengths are in Å.

8.4.4 D/I analysis of intermolecular (4+2) and (3+2) cycloadditions

To understand the intrinsic preference for the (3+2) cycloaddition, we analyzed the two transition structures using the distortion/interaction model. The distortion/interaction model$^{24\text{a}}$, or the activation strain model$^{24\text{b}}$, has recently been used to explain the reactivities and selectivities of cycloadditions in bioorthogonal chemistry$^{25}$, materials chemistry$^{26}$, and organic synthesis$^{27-28}$. For each reaction, the transition state is separated into two distorted fragments (diazo compound and diene) followed by single-point energy calculations on each fragment. The difference in energy between the distorted fragments and optimized ground-state geometries is the distortion energy of the diazo compound ($\Delta E_{\text{d}^\ddagger}^{\text{diazo}}$) and the diene ($\Delta E_{\text{d}^\ddagger}^{\text{diene}}$). The TS interaction energy ($\Delta E_{\text{int}^\ddagger}$) is the difference between the activation energy and the distortion energy ($\Delta E_{\text{int}^\ddagger} = \Delta E_{\text{activation}} - (\Delta E_{\text{d}^\ddagger}^{\text{diazo}} + \Delta E_{\text{d}^\ddagger}^{\text{diene}})$).
$\Delta E^\dagger - \Delta E_d^\dagger$). The results in Figure 8.5 show that the distortion energy of TS-(3+2) is similar to that of TS-(4+2). However, the interaction energy (-18.8 kcal/mol) is more favorable for TS-(3+2) compared to that of TS-(4+2) (-11.9 kcal/mol). In order to understand the difference, we have analyzed the interaction energies by FMO theory.

**Figure 8.5.** Distortion, interaction, and activation energies for the TS-(4+2) and TS-(3+2) (green: distortion energy of diene, blue: distortion energy of diazo compound, red: interaction energy, black: activation energy, in kcal/mol).

### 8.4.5 Frontier molecular orbital (FMO) analysis

The energies of relevant frontier orbitals were calculated with HF/6-31G(d) based on M06-2X/6-31G(d)-optimized transition state geometries. Figure 8.6 shows the HOMO and LUMO of each reactant distorted into its transition state geometry, for the (4+2) cycloaddition and the (3+2) cycloaddition. In each case, the LUMO$_{\text{diazo}}$ 1 or 1', HOMO$_{\text{diene}}$ 2 or 2' has the smaller HOMO–LUMO gap. While diazoalkanes are normally nucleophilic, the electron-withdrawing carbonyl group makes the diazo compound more electrophilic. Interestingly, in spite of the smaller FMO gap for the (4+2) cycloaddition, the (3+2) cycloaddition has a lower energy barrier and greater interaction energy (see Figures 8.4 and 8.5). We hypothesize this is due to the superior dipole LUMO-alkene HOMO orbital overlap.
Figure 8.6. FMO diagram for the distorted reactants of (4+2) and (3+2) cycloaddition reactions. HF/6-31G(d)//M06-2X/6-31G(d) computed orbital energies.

Figure 8.7 shows qualitatively that there is very large overlap of the 1,3-dipole LUMO (B1') with HOMO (A2') of the diene for the (3+2) cycloaddition. This is mainly due to the absence of a node in the alkene part of the HOMO, and the fact that the middle orbital of the dipole LUMO is small and does not interfere with orbital overlap. By contrast, the N=N LUMO of the dipole and the HOMO of the diene contain nodes, and while primary interactions are positive, the secondary overlap represented by the dashed lines are negative, leading to reduced overlap.
Figure 8.7. FMO diagram showing overlap between A2 and B1, and A2’ and B1’.

### 8.4.6 Origin of the preference for the intramolecular (4+2) cycloaddition to the diazo group

We have explained why the (3+2) cycloaddition will be favored in the intermolecular analog of the reaction studied here. This preference is overcome in the intramolecular reaction where the diene and diazo units are tethered by an ester linkage. This tether is highly distorted in the transition state leading to the (3+2) reaction, and the (4+2) reaction is consequently preferred. As we showed in Figure 8.1, the stabilizing conjugation of the ester with the diazoalkane is destroyed in TS-(3+2)-E and the normally planar ester is also distorted significantly from planarity. These distortions are absent in the (4+2) pathway, which is consequently preferred in the intramolecular reaction.

### 8.5 Conclusion

We have explored diazoester-diene cycloadditions, and found there is an intrinsic preference for diazoesters and dienes to give the 1,3-dipolar cycloaddition. The Diels–Alder reaction of a diene across the N=N bond of a diazoalkene is unfavorable and endergonic. However, the reaction of (E)-1 favors the intramolecular (4+2) across the diazo N=N bond,
because of the lack of strain in the tether and the strong orbital interactions. The $Z$-isomer of the diene is inert to both (3+2) and (4+2) cycloadditions due to the distortion of the tether required to achieve the transition state geometry. The reactivities of intermolecular cycloadditions are controlled by interaction energies. The intramolecular cycloaddition favors the hetero-DA reactions to the diazo N=N bond because of the high distortion energy of the tethering ester in the (3+2) cycloaddition.
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