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Authors
Patel, Ashay
Chen, Zhuo
Yang, Zhongyue
et al.

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Dynamically Complex [6+4] and [4+2] Cycloadditions in the Biosynthesis of Spinosyn A

Ashay Patel[†,‖], **Zhuo Chen**[‡,‖], **Zhongyue Yang**[†], **Osvaldo Gutiérrez**[†,⊥], **Hung-wen Liu**[§], **K. N. Houk**[*,†], and **Daniel A. Singleton**[*,‡]

[†]Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095-1569, United States

[‡]Department of Chemistry, Texas A & M University, College Station, Texas 77843-3255, United States

[§]College of Pharmacy and Department of Chemistry, University of Texas at Austin, Austin, Texas 78712-1224, United States

Abstract

SpnF, an enzyme involved in the biosynthesis of spinosyn A, catalyzes a transannular Diels–Alder reaction. Quantum mechanical computations and dynamic simulations now show that this cycloaddition is not well described as either a concerted or stepwise process, and dynamical effects influence the identity and timing of bond formation. The transition state for the reaction is ambimodal and leads directly to both the observed Diels–Alder and an unobserved [6+4] cycloadduct. The potential energy surface bifurcates and the cycloadditions occur by dynamically stepwise modes featuring an “entropic intermediate”. A rapid Cope rearrangement converts the [6+4] adduct into the observed [4+2] adduct. Control of nonstatistical dynamical effects may serve as another way by which enzymes control reactions.

Despite the prominence of the Diels–Alder reaction in synthetic chemistry, these cycloadditions only rarely play a role in the biosynthesis of natural products.[1] Enzyme-catalyzed Diels–Alder reactions have been particularly elusive, and their existence remained in doubt[2] some 70 years after Diels and Alder’s initial discovery.[3] More recent observations have suggested a role for enzymatic Diels–Alder reactions in the biosynthesis of a number of natural products.[4] The strongest evidence for a stand-alone Diels–Alderase has recently been presented by Liu et al. for SpnF, an enzyme that catalyzes the key transannular [4+2] cycloaddition of **4** to **5** in the biosynthesis of spinosyn A (Scheme 1).[4d]

[*]Corresponding Authors: houk@chem.ucla.edu. singleton@chem.tamu.edu.

[‖]**Author Contributions**

A.P. and Z.C. contributed equally.

[⊥]**Present Address**

O.G.: Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104-6323, USA

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Supporting Information

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Computational data, including Cartesian coordinates of transition states and minima, their zero-point energy and thermal corrections, and the imaginary frequencies of transition states (PDF)

The mechanism of the Diels–Alder reaction has been a topic of scholarly discussion and passionate debate,[5] since the discovery of this reaction in 1928. In contrast, the mechanistic nature of enzymatic Diels–Alder reactions is much less well understood. We now provide a complete description of the mechanism of the nonenzymatic Diels–Alder reaction of macrolactone **4**, an intermediate in the biosynthesis of spinosyn A (**1**) using quantum mechanical computations and molecular dynamics simulations. A previous theoretical study of this reaction by Smentek and Hess indicated that the SpnF-catalyzed cycloaddition was "concerted, [but] highly asynchronous".[6] Diels–Alder reactions involve the formation of cyclohexenes from dienes and alkenes, but the timing of bond formation may vary, from concerted synchronous, where both bonds form at the same time in a single transition state, to concerted asynchronous, where one bond is formed more completely than the other in the transition state.[7] The mechanism of the Diels–Alder reaction may also be stepwise with the two bonds being formed in different transition states separated by an intermediate diradical or zwitterion. Yet another mechanism features a single ambimodal transition state[8] that leads to two or more products. We show here that the tricyclic macrolactone **5** (Scheme 1) forms via such an ambimodal transition state, a transition state that also directly leads to a previously undetected [6+4] cycloaddition product that is readily converted to the observed Diels–Alder product.

QM calculations were performed using the SMD(H_2O)/M06-2X/def2-TZVPP//B3LYP-D3(BJ)//6-31+G(d,p) level of theory. Details are provided in the Supporting Information. The lowest energy gas-phase transition state located computationally for the reaction of **4** is **TS7** (Figure 1). The [4+2] cycloadduct formed via this transition state is the only product observed experimentally; transition structures leading to stereoisomers of **5** (or **6**) were computed to be at least 3 kcal mol^{-1} higher in energy. Remarkably, the structure of **TS7** is not that of a simple asynchronous Diels–Alder transition state. Rather, it is "bis-pericyclic",[9] or, more generally, ambimodal.[8] A bis-pericyclic transition state features two sets of stabilizing cyclic aromatic orbital interactions that can give two different pericyclic reaction products, here [6+4] and [4+2] cycloadditions;[10] the transition state features *three* partially formed σ-bonds, C^7–C^{11}, C^4–C^{12}, and C^2–C^{14}, with forming bond distances of 1.95, 3.00, and 2.98 Å, respectively. Predicted in 1965,[10a] [6+4] cycloadditions were soon detected experimentally;[11] many examples have been described in the past 50 years.[12]

The free-energy landscape for the cyclization of **4** is illustrated in Figure 2. The bis-pericyclic transition state **TS7** is 28 kcal mol^{-1} higher in energy than the reactant. Following **TS7**, the reaction path "bifurcates" in two directions with one direction leading to the formation of the expected Diels–Alder adduct **5** and the other leading to the [6+4] cycloadduct **6**. The steepest-decent path from **TS7** along the potential energy surface in mass-weighted coordinates (the minimum energy path, or MEP) leads to the [6+4] adduct **6**. The [6+4] adduct **6** is 13 kcal mol^{-1} higher in free energy than the Diels–Alder, and is predicted to undergo a facile Cope rearrangement to afford the Diels–Alder adduct.

The product outcomes of reactions featuring bis-pericyclic transition states and bifurcating potential energy surfaces cannot be predicted with conventional transition state theory, because dynamical effects control the selectivity of the reaction. We performed molecular dynamics trajectory calculations to determine the competition between formation of the

[6+4] and [4+2] products. Quasi-classical direct-dynamics simulations[13] on a B3LYP-D2/6-31G(d) energy surface[14] were performed. The trajectories were initiated within the potential energy surface near **TS7** and integrated in time in both the forward and backward directions until either **5** or **6** was formed or reactant **4** was reformed.

Trajectories passing through **TS7** end in one of *three* ways. Out of 353 trajectories simulated, 223 (63%) afford the [6+4] product **6**, 88 (25%) afford the Diels–Alder adduct **5**, and 42 (12%) *recross*, fully forming the C^7–C^{11} bond before passing back through the transition state to reform the starting material. These simulations predict that [6+4] and [4+2] adducts are initially formed in a 2.5:1 ratio.

The time required for formation of either **5** or **6** is unusually long compared to the time required for the formation of other Diels–Alder adducts. The median times for formation of **5** and **6** starting from the initialization at **TS7**, defined as the time required for both new σ bonds to reach carbon–carbon distances of 1.7 Å, were 171 and 197 fs, respectively. Over 20% of the trajectories require longer than 300 fs to afford **6**. Simple Diels–Alder reactions typically require less than 50 fs to form products from the transition state.[15] Another way to determine this time is to assess the time required by trajectories to cross the "transition zone", which is defined as the set of geometries thermally accessible at the transition state assuming the harmonic frequencies of the transition state. When this is done, the median times in the transition zone for trajectories leading to **5** and **6** are 130 and 127 fs, respectively, compared to 50–95 fs in other simpler Diels–Alder reactions.[15] Finally, the time gap between formation of the first new σ bond in the cycloadduct (the C^7–C^{11} bond) and formation of the second new σ bond (either C^4–C^{12} for **5** or C^2–C^{14} for **6**, defined by interatomic distances <1.7 Å) is exceptionally long, with median times of 173 and 139 fs for **5** and **6**, respectively. For comparison, the Diels–Alder reactions of simple symmetrical reactants (e.g., the reaction of ethylene and butadiene) have median time gaps of shorter than 5 fs while unsymmetrical cycloaddends undergo cycloaddition with longer median time gaps between 10 to 25 fs. Thus, the cycloaddition of **4** is predicted to be energetically concerted, but dynamically and bonding stepwise.[15]

The simulations illustrate the dynamical nature of the cycloaddition. Figure 3a shows a plot of three different types of trajectories in three dimensions, defined by the C^7–C^{11}, C^4–C^{12}, and C^2–C^{14} interatomic distances, while Figure 3b shows a plot of 30 randomly chosen trajectories. Reactions begin with the approach of C^7 and C^{11} toward one another, passing through the cycloaddition transition state when the C^7–C^{11} distance is about 1.97 Å, then fully forming the C^7–C^{11} bond. The structure that results is loosely delineated by C^4–C^{12} and C^2–C^{14} distances between 2.8 and 3.2 Å (between the ovals in Figure 3b). The structure tends to persist for a few bond vibrations, and it may undergo three distinguishable reactions, i.e., formation of the Diels–Alder adduct **5**, formation of the [6+4] adduct **6**, or transition state recrossing to the reactant **4**. From these properties, the structure is best understood as an intermediate, even though there is no potential-energy barrier for the formation of either of the C^4–C^{12} and C^2–C^{14} bonds. Trajectories show that the macrolactone persists in the region of the potential energy surface, briefly, because the route to the two products must pass through a dynamical bottleneck. In qualitative terms, the C^4–C^{12} and C^2–C^{14} atom pairs are energetically attracted, but their bond formation requires the

entropically disfavored event that one or both of the pairs of atoms specifically approach each other.

A more quantitative, statistical approach to understanding the nature of the mechanism was pursued by applying canonical variational transition state theory (VTST).[16] With VTST, two variational transition states, **VTS9** corresponding closely to the potential-energy saddle point **TS7** and **VTS10** (Figure 4), which occur after the C^7–C^{11} bond is fully formed, were located. Between **VTS9** and **VTS10**, the MEP exhibits a shallow minimum in the generalized free energy. This minimum can formally be considered an intermediate[17] that arises because the formation of either the C^4–C^{12} or C^2–C^{14} bonds reduces the flexibility of the macrolactone in a way that is entropically unfavorable. The intermediate is delimited at **VTS10** by this entropic barrier. Such variational intermediates, which only appear on a free energy surface, have been suggested before by Doubleday in his studies of the tetramethylene biradical.[18] Overall, the statistical picture agrees well with the qualitative picture derived from the trajectories.

Mechanistic chemistry that divides reactions into concerted versus two-step is inadequate to describe the complex cycloaddition of **4**. There is no opportunity for the “intermediate” to be trapped or lose its stereochemistry; the intermediate itself has no potential-energy minimum and has no parallel in the ions or radicals of ordinary reactive-intermediate chemistry. In contrast to the usual idea of a concerted Diels–Alder mechanism, the cycloaddition of **4** involves a very shallow free-energy minimum along the reaction pathway, and can ultimately evolve along multiple pathways, a standard indicator of an intermediate. Similar concepts have been proposed by John Baldwin (bonding stepwise, but energetically concerted)[19] and by Raymond Firestone (diradical intermediates with lifetimes too short for bond rotation).[20] The potential energy and free energy surfaces differ, and give different classifications of the mechanism of the cycloaddition as concerted or stepwise.

The mechanism of the SpnF-catalyzed cycloaddition of **4** is currently unknown, and it need not be identical to the uncatalyzed gas-phase mechanism. Nonetheless, these data beg the question: Can an enzyme control the outcome of an ambimodal reaction by altering the energy surface such that the steepest downhill path from the transition state leads to the “desired” product(s)? Tantillo et al. have suggested that this sort of dynamic selection plays a role in terpene biosynthesis[21a] Such possibilities are entirely distinct from the lowering of reaction barriers that is assumed to completely define catalysis, but they are recognizable when the dynamic complexity of real reactions. Hoye et al. have demonstrated that a biomimetic Diels–Alder reaction is ambimodal.[21b] Recently, a related ambimodal [6+4] cycloaddition was implicated in the biosynthesis of heronamide A, but in this case the [6+4] adduct was found to be more thermodynamically stable than the isomeric Diels–Alder product.[21c]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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14. The energies determined at this level of theory are similar to those calculated using the B3LYP-D3(BJ)/6-31+G(d,p) model chemistry (ca. ±4 kcal mol^{-1}). See Supporting Information.
15. We have defined a "dynamically stepwise" mechanism as one in which bond formation has a time gap of 60 fs or greater. See ref 17.
16. The resulting variational transition states are dynamical bottlenecks and correspond to maxima in the free energy along the MEP from reactant to product Truhlar DG, Garrett BC. Acc Chem Res. 1980; 13:440.

17. Computations suggest that the intermediate is neither a diradical nor a zwitterion, but rather a 10-π-electron aromatic species that must undergo nuclear vibrations in order form a second bond.
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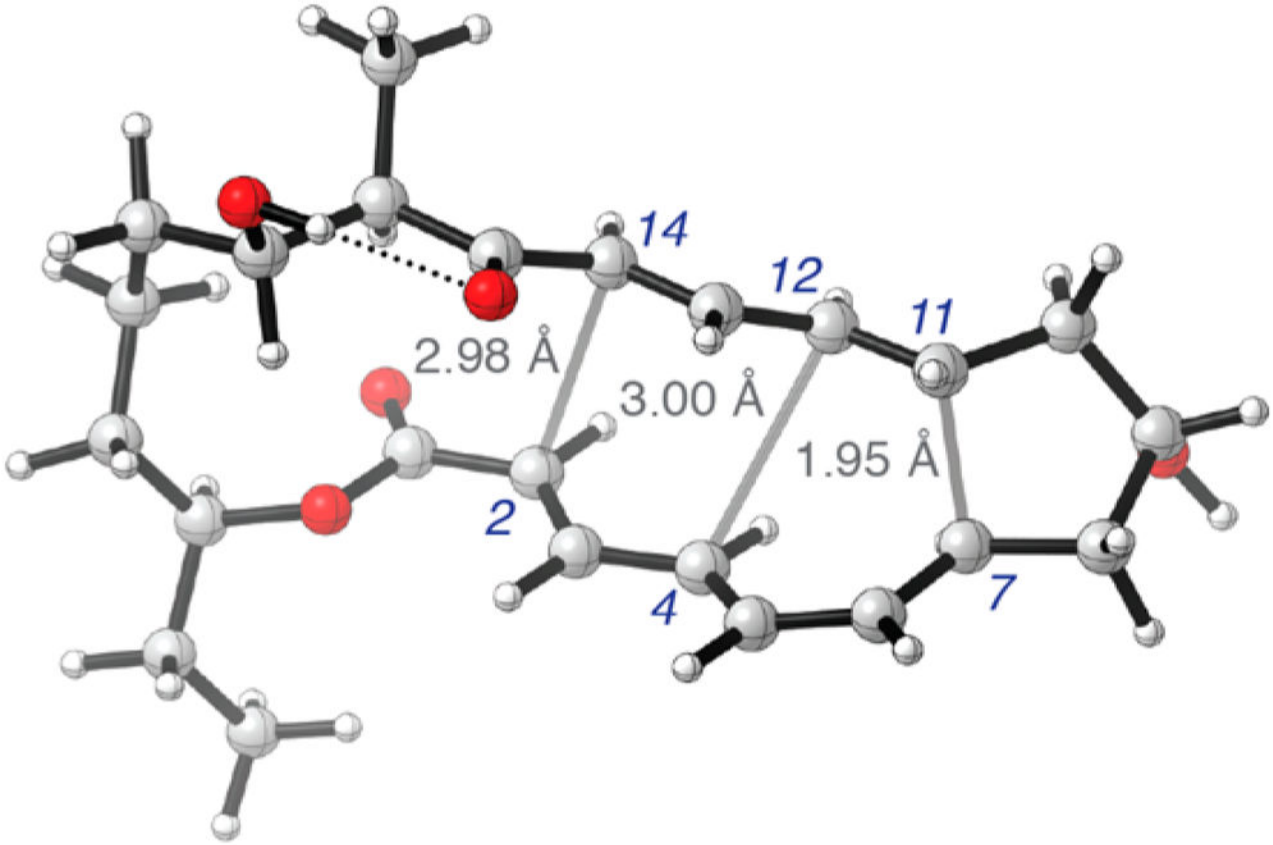


Figure 1.
"Ambimodal" transition structure **TS7**. The forming-bond distances (Å) are shown in gray.

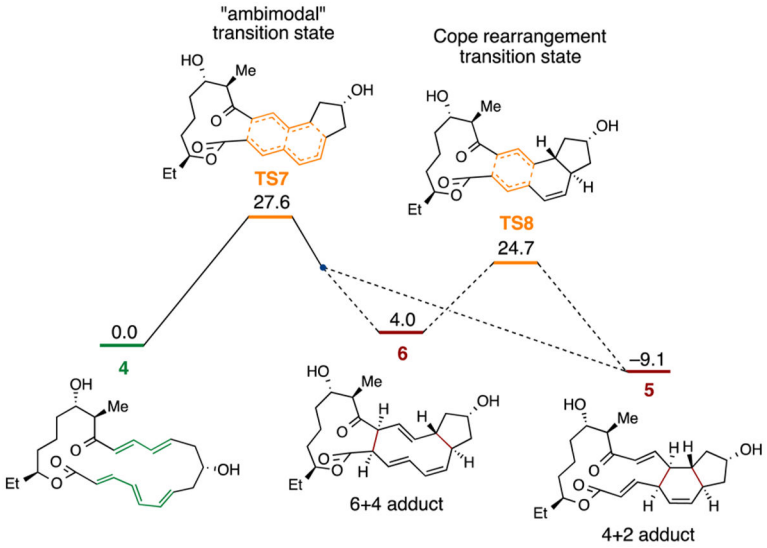


Figure 2.

Free energy diagram for the cycloaddition of **4**. Gibbs free energies in kcal mol^{-1}.

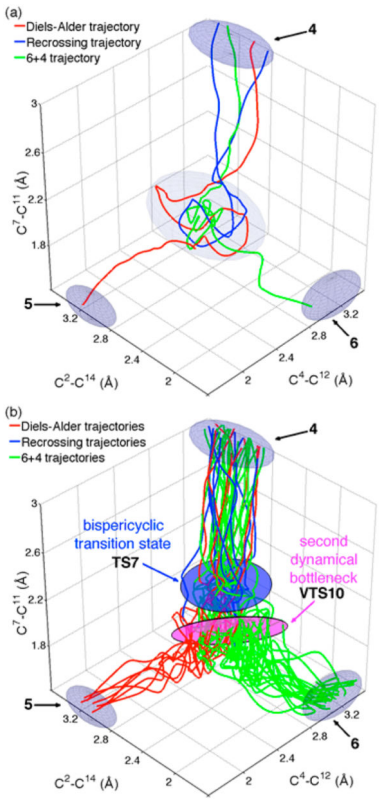


Figure 3.

Trajectory plots following the C–C interatomic distances: (a) three selected trajectories and (b) 30 randomly chosen trajectories.

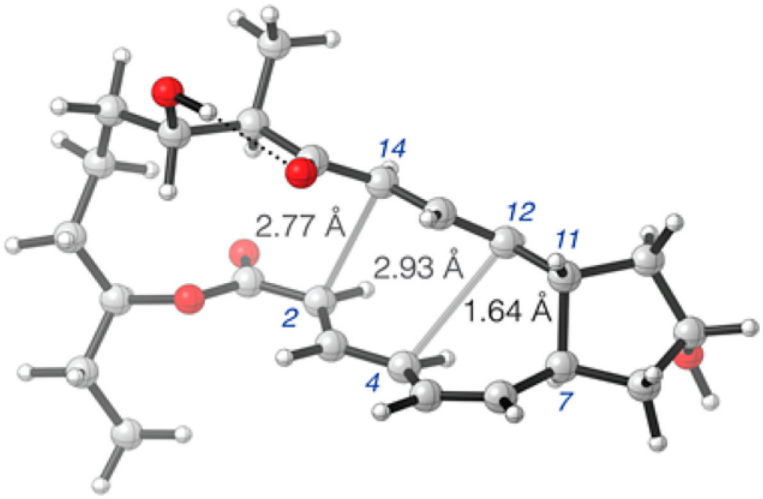
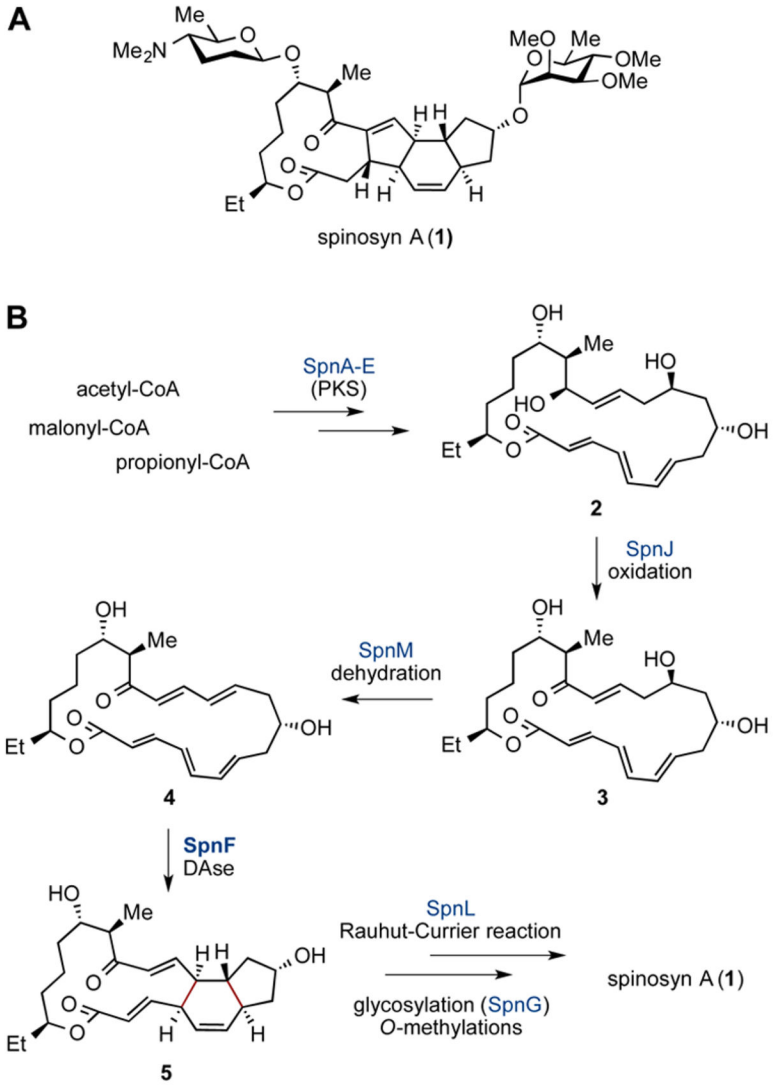


Figure 4.
Variational transition state (dynamical bottleneck **VTS10**) computed using B3LYP-D2/6-31G(d).



Scheme 1.
Structure (A) and Biosynthesis (B) of Spinosyn A