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How an Enzyme Might Accelerate an Intramolecular Diels−Alder Reaction: Theozymes for the Formation of Salvileucalin B

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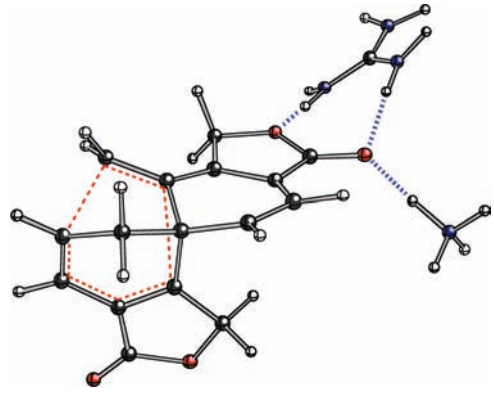
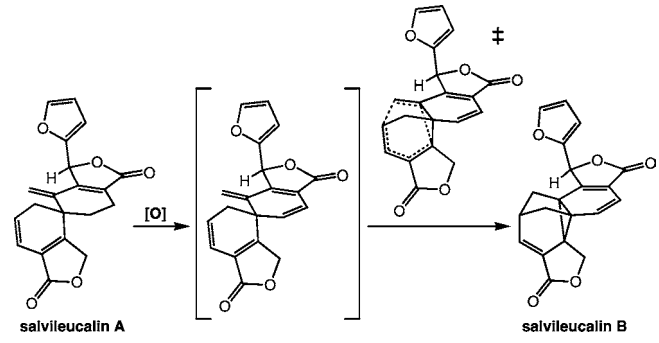
ABSTRACT



Quantum chemical calculations reveal how functional group arrays present in known enzyme active sites could accelerate an intramolecular Diels−Alder reaction proposed to occur during the biosynthesis of a complex terpenoid natural product.

Salvileucalin B (Scheme 1) is a polycyclic terpenoid isolated from the sage bush *Salvia leucantha*.[1] Given that salvileucalin A was also isolated from this plant, it was proposed that salvileucalin B could arise from salvileucalin A via oxidation followed by an intramolecular [4 + 2] cycloaddition (Diels−Alder reaction; Scheme 1).[1] 6-Membered rings in many natural products have been proposed to arise from biosynthetic Diels−Alder reactions.[2] Nonetheless, firm evidence for the existence of Diels−Alderase enzymes is scant.[2,3] Several catalytic antibodies that promote Diels−Alder reactions have been isolated and characterized, but these do

Scheme 1



(1) (a) Aoyagi, Y.; Yamazaki, A.; Nakatsugawa, C.; Fukaya, H.; Takeya, K.; Kawaucji, S.; Izumi, H. *Org. Lett.* **2008**, *10*, 4429–4432. (b) For a recent synthetic approach to the salvileucalins, see: Levin, S.; Nani, R. R.; Reisman, S. E. *Org. Lett.* **2010**, *12*, 780−783.

(2) Reviews: (a) Kelly, W. L. *Org. Biomol. Chem.* **2008**, *6*, 4483–4493. (b) Stocking, E. M.; Williams, R. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 3078–3115. (c) Oikawa, H. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 537–554. (d) Oikawa, H.; Tokiwano, T. *Nat. Prod. Rep.* **2004**, *21*, 321–352. (e) Pohnert, G. *ChemBioChem* **2001**, *2*, 873–875.

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not produce natural products.[4] Herein we describe quantum chemical calculations aimed at assessing the inherent feasibility of the proposed salvileucalin B-forming Diels−Alder reaction, as well as the potential for an enzyme to accelerate this reaction using simple, biologically reasonable, noncovalent interactions.

The barrier for the enzyme-free cycloaddition was computed using a variety of different quantum chemical methods[5,6] and a model system lacking only the furan ring of the natural product (Figure 1, **A**).[7] At the B3LYP/6-31G(d) level, a level of theory frequently used for computing barriers for [4 + 2] cycloadditions,[8] this reaction has a barrier of 28 kcal/mol. It is likely that this barrier is an overestimate, however, given the results of previous theoretical studies on cycloadditions and other hydrocarbon cyclization reactions.[6,8] Our single point calculations at the mPW1PW91/6-31G(d) and MP2/6-31G(d) levels do predict lower barriers (25.0 and 19.2 kcal/mol, respectively; Figure 1, **A**). Previous work suggests that MP2 may lead to underestimates of such barriers,[8] so we suspect that our mPW1PW91 barrier is most reasonable. The inclusion of solvation, here using a continuum dielectric model[5] for $CHCl_3$ as an admittedly crude approximation of an enzyme active site,[9] had only a small effect on the barrier, lowering it slightly (by <1 kcal/mol; Figure 1, **A**).

To explore the effects of various substructures of the salvileucalin B framework on the magnitude of this barrier, a series of truncated models were explored (Figure 1, **B**−**F**). Calculations on model **B** show that if the cycloaddition was attempted before oxidation (i.e., from salvileucalin A itself),

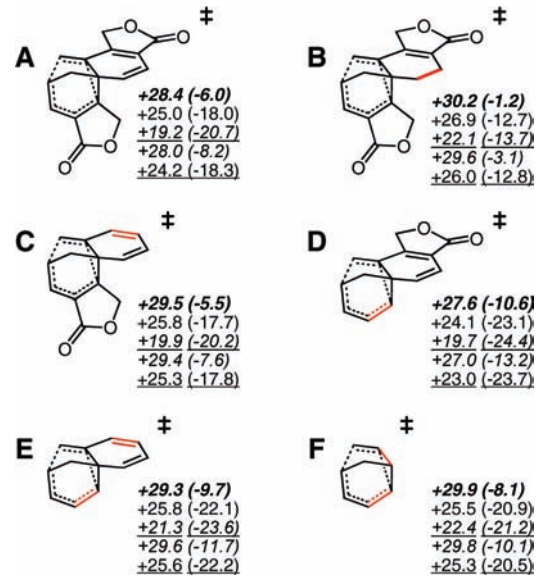

Figure 1. Activation barriers and reaction energies (parentheses) for models of salvileuaclin A/B. All energies are in kcal/mol. Bold italics: B3LYP/6-31G(d)+0.9806ZPE; normal text: mPW1PW91/6-31G(d)//B3LYP/6-31G(d); underlined italics: MP2/6-31G(d)//B3LYP/6-31G(d); simple italics: CPCM($CHCl_3$,UAKS)-B3LYP/6-31G(d)//B3LYP/6-31G(d); simple underlined: CPCM($CHCl_3$,UAKS)-mPW1PW91/6-31G(d)//B3LYP/6-31G(d).

(3) Macrophomate synthase was touted by some as the first well-characterized Diels−Alderase enzyme, but evidence has been accumulated that indicates that the cyclization catalyzed by this enzyme is stepwise, and therefore not truly pericyclic. See, for example: (a) Guimarães, C. R. W.; Udier-Blagović, M.; Jorgensen, W. L. *J. Am. Chem. Soc.* **2005**, *127*, 3577–3588. (b) Ose, T.; Watanabe, K.; Mie, T.; Honma, M.; Watanabe, H.; Yao, M.; Oikawa, H.; Tanaka, I. *Nature* **2003**, *422*, 185–189.

(4) Keinan, W., Ed. *Catalytic Antibodies*; Wiley-VCH: Weinheim, Germany, 2005.

(5) GAUSSIAN03 (Frisch, M. J. et al., *GAUSSIAN03*, Revision D.01; Gaussian, Inc., Pittsburgh, PA, 2003, full reference in the Supporting Information) was employed for all calculations. All geometries were optimized at the B3LYP/6-31G(d) level of theory: Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372–1377. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B: Solid State* **1988**, *37*, 785–789. Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623–11627. All stationary points were characterized as minima or transition state structures by analyzing their vibrational frequencies. Intrinsic reaction coordinate (IRC) calculations (Gonzalez, C.; Schlegel, H. B. *J. Phys. Chem.* **1990**, *94*, 5523–5527. Fukui, K. *Acc. Chem. Res.* **1981**, *14*, 363–368) were used to further characterize the identity of selected transition state structures (for the parent system **A** and for system **K** to assure that proton transfer did not occur). Single point energy calculations using mPW1PW91/6-31G(d)[6] and MP2/6-31G(d) (Møller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618–622) were performed on some optimized B3LYP/6-31G(d) geometries, as described in the text. All reported energies from B3LYP/6-31G(d) calculations include zero point energy corrections from frequency calculations, scaled by 0.9806: Scott, A. P.; Radom, L. *J. Phys. Chem.* **1996**, *100*, 16502–16513. For some systems, the effects of solvent ($CHCl_3$ [ε 4.9]) were modeled using CPCM calculations (with UAKS radii), a self-consistent reaction field (SCRF) method: Barone, V.; Cossi, M. J. *J. Phys. Chem. A* **1998**, *102*, 1995–2001. Barone, V.; Cossi, M.; Tomasi, J. *J. Comput. Chem.* **1998**, *19*, 404–417. Takano, Y.; Houk, K. N. *J. Chem. Theor. Comput.* **2005**, *1*, 70–77. Single-point energies are reported throughout the text, but reactants and transition state structures for two systems—**G** and **K**—were also optimized at the CPCM($CHCl_3$,UAKS)-B3LYP/6-31G(d) level. For system **G**, the largest geometric differences between transition state structures optimized in the gas phase and in solvent were found, not surprisingly, in the O···H−O substructures: the $O_{carbonyl}$···H distance was 0.11 Å shorter and the O_{ester}···H distance was 0.12 Å longer in solvent than in the gas phase; changes to the bonds that form and break during the Diels−Alder reaction, as well as changes to the O−H bonds in the water molecule, were all well less than 0.01 Å. The barrier computed with structures optimized in solvent was 27.0 kcal/mol, ~0.4 kcal/mol lower than that computed with single-point energies (see Figure 2). For system **K**, full optimization of the transition state structure in solvent failed, since, after an initial period of structural optimization, the calculation consistently oscillated between structures extremely close in geometry and differing in energy by less than 0.02 kcal/mol. Using the structure with lower energy of these two and the fully optimized reactant, an upper limit for the barrier of 25.8 kcal/mol (not zero-point energy corrected) was computed; this value is only ~0.2 kcal/mol lower than that calculated with single-point energies (see Figure 2). Here, the largest geometric differences between the transition state structures optimized in the gas phase and in solvent were found in the O···H−N substructure: the O···H distance was 0.16 Å longer and the H−N distance was 0.06 Å shorter in solvent than in the gas phase; changes to the bonds that form and break during the Diels−Alder reaction were all less than 0.025 Å. Thus, using solvent single-point energies to compute barriers seems reasonable. The structural drawing in Figure 3 was produced using Ball & Stick (Müller, N.; Falk, A. *Ball & Stick V.3.7.6*, molecular graphics application for MacOS computers; Johannes Kepler University: Linz, Austria, 2000).

(6) Matsuda, S. P. T.; Wilson, W. K.; Xiong, Q. *Org. Biomol. Chem.* **2006**, *4*, 530–543.

(7) The furan ring was replaced by a hydrogen atom in order to reduce the time required for computations (this ring system would add five non-hydrogen atoms and some conformational flexibility). We feel that this approach is reasonable since the furan ring is not directly conjugated to any of the π-systems present in the salvileucalins.

(8) (a) Ess, D. H.; Houk, K. N. *J. Phys. Chem. A* **2005**, *109*, 9542–9553. (b) Guner, V. A.; Khuong, K. S.; Houk, K. N.; Chuma, A.; Pulay, P. *J. Phys. Chem. A* **2004**, *108*, 2959–2965. (c) Guner, V.; Khuong, K. S.; Leach, A. G.; Lee, P. S.; Bartberger, M. D.; Houk, K. N. *J. Phys. Chem. A* **2003**, *107*, 11445–11459. (d) Wiest, O.; Montiel, D. C.; Houk, K. N. *J. Phys. Chem. A* **1997**, *101*, 8378–8388.

(9) For discussion and leading references, see: (a) Lee, J. K.; Tantillo, D. J. *Adv. Phys. Org. Chem.* **2003**, *38*, 183–218. (b) Shutz, C. N.; Warshel, A. *Proteins Struct. Funct. Genet.* **2001**, *44*, 400–417. (c) Simonson, T.; Brooks, C. L., III. *J. Am. Chem. Soc.* **1996**, *118*, 8452–8458.

a higher barrier would be expected. Calculations on model **C** (compare with **A**) indicate that the presence of the "top" lactone has a slight accelerating effect on the reaction, as expected for an electron-withdrawing group attached to a dienophile. Calculations on model **D** indicate that the presence of the "bottom" lactone has a decelerating effect, which is not surprising for a "normal electron demand" Diels−Alder reaction (an enzyme could, in principle, deactivate the "bottom" lactone by reversibly adding an active site nucleophile to it). The effect of the top lactone is also seen in the comparison of our results obtained using models **D** and **E**, while the effect of the bottom lactone appears to be negligible in the absence of the top lactone (compare **C** and **E**). Calculations on model **F** provide a measure of the inherent barrier for an intramolecular Diels−Alder reaction that produces the tricyclic system at the core of salvileucalin B, revealing that the conjugated system appended to the dienophile (sans appended lactone; compare **F** to **E**) has only a very small, if any, effect on the barrier. Overall, the effects of the substituents on the cycloaddition barrier amount to less than 3 kcal/mol,[10] however.

Given that the cycloaddition barrier is likely too high (ca. 25 kcal/mol) for the reaction to proceed efficiently under biological conditions without assistance, we examined whether or not the barrier could be reduced by noncovalent interactions of the types that could occur in an enzyme active site. In short, can hydrogen bonding to the "top" lactone with biological binding motifs enhance its electron-withdrawing ability[11] enough to lower the barrier to a biologically reasonable range (i.e., less than approximately 20 kcal/mol[12])? To explore this issue, a variety of "theozymes"[13] were examined (Figure 2). Complexation with a water molecule (**G**; also a simple model of a serine, threonine, or tyrosine side chain) lowered the cycloaddition barrier by 0.7 kcal/mol or less. Binding to the lactone carbonyl with two amides (**H**; a model of a typical "oxyanion hole" found in ester hydrolyzing enzymes[14]) lowered the barrier by ca. 1 kcal/mol. Use of positively charged residue models—either a guanidinium (**I**; a model of protonated arginine), an imidazolium (**J**; a model of protonated histidine), or an ammonium (**K**, a model of protonated lysine)—led to more significant reductions in the cycloaddition barrier ranging from ∼2 to 6 kcal/mol. Finally, a theozyme consisting of both a guanidinium and an ammonium (**L**; a motif that has been observed previously in the active sites of catalytic antibodies that hydrolyze esters;[15] see Figure 3 for the

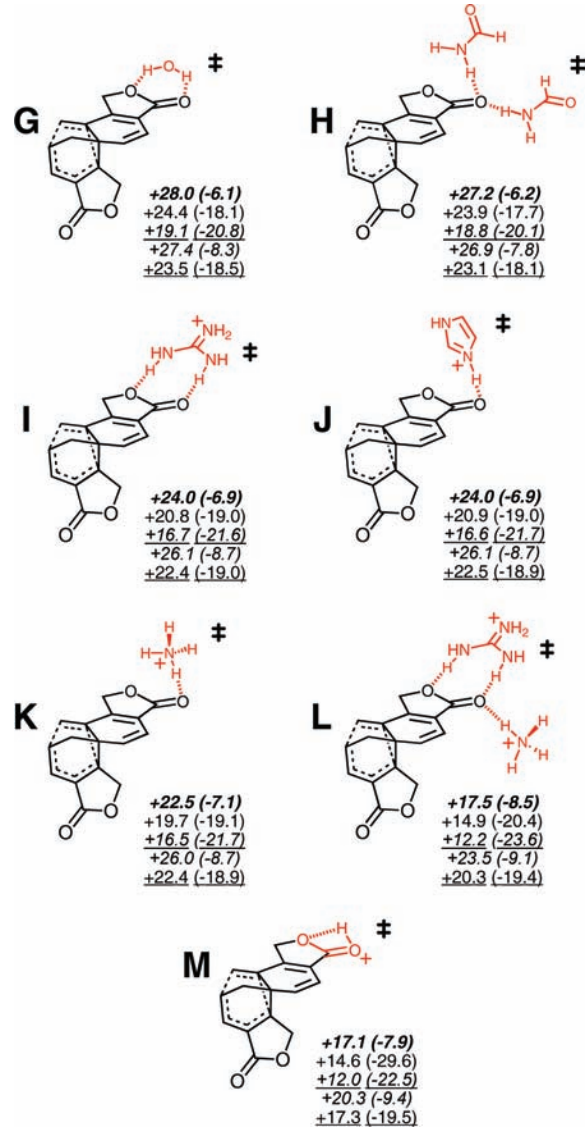

Figure 2. Activation barriers and reaction energies (parentheses) for theozyme-bound (**G**−**L**) and protonated (**M**) analogues of **A**. All energies are in kcal/mol. Bold italics: B3LYP/6-31G(d)+0.9806ZPE; normal text: mPW1PW91/6-31G(d)//B3LYP/6-31G(d); underlined italics: MP2/6-31G(d)//B3LYP/6-31G(d); simple italics: CPCM($CHCl_3$,UAKS)-B3LYP/6-31G(d)//B3LYP/6-31G(d); simple underlined: CPCM($CHCl_3$,UAKS)-mPW1PW91/6-31G(d)//B3LYP/6-31G(d).

(10) Note that all of the reactions in Figure 1 are predicted to be exothermic; trading π-bonds for σ-bonds counteracts the increase in strain upon cycloaddition.

(11) For leading references on related examples, see: (a) Tantillo, D. J. *Angew. Chem., Int. Ed.* **2009**, *48*, 31–32. Selected studies involving [4 + 2] reactions: (b) Linder, M.; Brinck, T. *Org. Biomol. Chem.* **2009**, *7*, 1304–1311. (c) Anderson, C. D.; Dudding, T.; Gordillo, R.; Houk, K. N. *Org. Lett.* **2008**, *10*, 2749–2752. (d) Zhang, X.; DeChancie, J.; Gunayadin, H.; Howdry, A. B.; Clemente, F. R.; Smith, A. J. T.; Handel, T. M.; Houk, K. N. *J. Org. Chem.* **2008**, *73*, 889–899. (e) Cannizzaro, C. E.; Ashley, J. A.; Janda, K. D.; Houk, K. N. *J. Am. Chem. Soc.* **2003**, *125*, 2489–2506. (f) Zhang, X.; Deng, Q.; Yoo, S. H.; Houk, K. N. *J. Org. Chem.* **2002**, *67*, 9043–9053.

(12) For leading references, see: (a) Warshel, A.; Sharma, P. K.; Kato, M.; Xiang, Y.; Liu, H.; Olsson, M. H. M. *Chem. Rev.* **2006**, *106*, 3210–3235. Table 1 of this review lists enzymatic reactions for which free energies of activation (bound reactant(s) to bound rate-determining transition state structure) have been determined, and these range from 11 to 19 kcal/mol. (b) Garcia-Viloca, M.; Gao, J.; Karplus, M.; Truhlar, D. G. *Science* **2004**, *303*, 186–195. (c) Snider, M. G.; Temple, B. S.; Wolfenden, R. *J. Phys. Org. Chem.* **2004**, *17*, 586–591. (d) Wolfenden, R.; Snider, M. J. *Acc. Chem. Res.* **2001**, *34*, 938–945.

(13) Reviews: (a) Tantillo, D. J.; Chen, J.; Houk, K. N. *Curr. Op. Chem. Biol.* **1998**, *2*, 743–750. (b) Tantillo, D. J.; Houk, K. N. Theozymes and Catalyst Design. In *Stimulating Concepts in Chemistry*; Wiley-VCH: Weinheim, Germany, 2000; pp 79−88. (c) Sousa, F. P.; Fernandes, P. A.; Ramos, M. J. *J. Phys. Chem. A* **2009**, *113*, 14231–14236.

(14) For leading references, see: (a) Ordentlich, A.; Barak, D.; Kronman, C.; Ariel, N.; Segall, Y.; Velan, B.; Shafferman, A. *J. Biol. Chem.* **1998**, *273*, 19509–19517. (b) Whiting, A. K.; Peticolas, W. L. *Biochemistry* **1994**, *33*, 552–561.

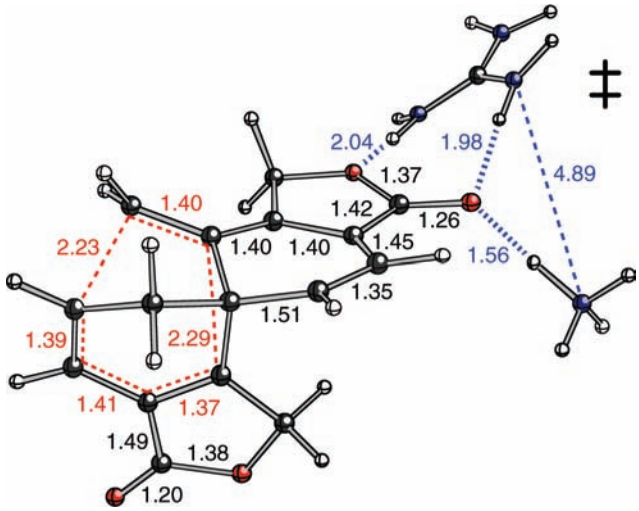

Figure 3. B3LYP/6-31G(d) optimized geometry of transition state structure for the intramolecular Diels−Alder reaction of model system **L**. Selected distances are given in Å.

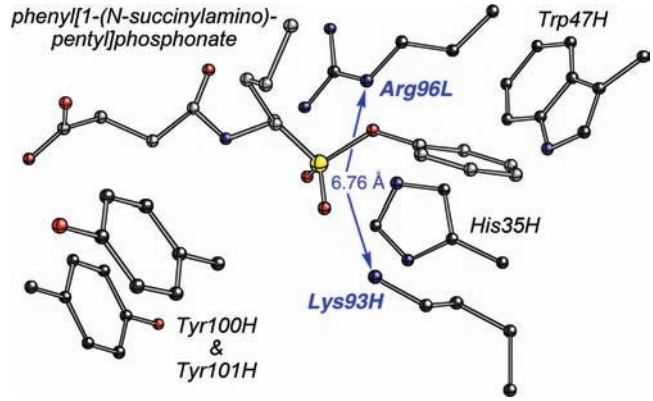

Figure 4. Crystallographically determined binding site of catalytic antibody 17E8 bound to an analogue of an ester hydrolysis transition state structure.[15c]

computed transition state structure and Figure 4 for a catalytic antibody binding site displaying this motif) led to an even greater reduction in the barrier—up to 11 kcal/mol in the gas phase, comparable to that of explicit protonation of the lactone (**M**). Solvation, however, washed out much of the effect of complexation. Nonetheless, the barrier predicted for model system **L** at the CPCM($CHCl_3$,UAKS)-mPW1PW91/6-31G(d)//B3LYP/6-31G(d) level of theory, the level that we consider to be most reasonable, is approximately 20 kcal/mol. This barrier is at the high end of the range of typical barriers for enzyme-catalyzed reactions.[12] $\pi-\pi$ or dispersion interactions could also possibly help to lower the cycloaddition barrier, but these types of interactions are difficult to model correctly, especially with density functional theory, and were not treated further here.

Thus, we suggest that noncovalent catalysis in the classic sense described by Pauling,[16] i.e., selective binding of the transition state structure over the reactant, is a feasible strategy for promoting this cycloaddition reaction in a biological (or possibly nonbiological[17]) setting. In that the theozymes considered herein consist of functional group arrays that are well-established as catalytic groups in known enzymes, in particular those that promote reactions of esters, it is not hard to envision a biological catalyst that could interact selectively with the "top" ester of the salvileucalins and promote a salvileucalin B-forming Diels−Alder reaction, nor is it difficult to imagine that such an enzyme could have evolved from, for example, an esterase. Although the small models described herein may not capture the full extent to which an enzyme can manipulate a Diels−Alder reaction, they do reveal some of the sorts of interactions that could contribute significantly to barrier-lowering.

Acknowledgment. We gratefully acknowledge UC Davis, the National Science Foundation's CAREER program (CHE-0449845), and the National Science Foundation's Partnership for Advanced Computational Infrastructure (CHE-030089, Pittsburgh Supercomputer Center) for support.

Supporting Information Available: Full computational details, including coordinates and energies for all structures, data on IRC calculations, and the full GAUSSIAN reference. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) (a) Tantillo, D. J.; Houk, K. N. *J. Comput. Chem.* **2002**, *23*, 84–95. (b) Tantillo, D. J.; Houk, K. N. *Chem. Biol.* **2001**, *8*, 535–545. (c) Zhou, G. W.; Guo, J.; Huang, W.; Scanlan, T. S.; Fletterick, R. J. *Science* **1994**, *265*, 1059–1064.

(16) Pauling, L. *Nature* **1948**, *161*, 707.

(17) Our results suggest that biomimetic syntheses of salvileucalins and related compounds might benefit from performing the Diels−Alder reaction prior to installing the "bottom" lactone and attempting to promote it with an organocatalyst or Lewis acid; representative examples of both are shown below (activation barriers and reaction energies (parentheses) for thioamide (**N**) and $Al(CH_3)_2Cl$ (**O**) complexed analogues of **D**;[11] all energies (B3LYP/6-31G(d)+0.9806ZPE) are in kcal/mol):