duction differs from most of these reactions in that the carbon–oxygen bond of the acetal group is not cleaved during the reaction.[17] Studies are under way to clarify the mechanism of the asymmetric induction as well as that of the carbocupration reaction.

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Supplementary Material Available: Structure determination and physical properties of the cyclopropanes (8 pages). Ordering information is given on any current masthead page.

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An Antibody-Catalyzed Bimolecular Diels–Alder Reaction

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There exist over 1500 known enzymes which carry out a vast array of chemical reactions with remarkable specificity and reaction rates. It is surprising then that there are no documented examples of enzyme-catalyzed pericyclic cycloaddition reactions,[1] yet these are among the most powerful and commonly used reactions in synthetic organic chemistry. The most important of these is the Diels–Alder reaction of a diene with a dienophile, which provides a straightforward and highly stereospecific route to cyclohexene derivatives. In contrast to the majority of enzyme-catalyzed reactions, this reaction is believed to typically proceed through a concerted transition state involving the simultaneous formation of carbon–carbon bonds within a cyclic array of interacting orbitals.[2] Given the importance of this reaction in organic chemistry and its novel mechanism, it was of interest to ask whether a "Diels–Alderase" enzymelike catalyst could be evolved from an antibody combining site.[3]

Generation of antibodies to a structure that mimics the pericyclic transition state for a Diels–Alder reaction should result in an antibody combining site that lowers the entropy of activation $\Delta S^{\ddagger}$ by binding both the diene and the dienophile in a reactive conformation. The idea of using antibodies as "entropic traps" to lower the translational and rotational activation entropy of a reaction has been realized in antibody-catalyzed Claisen rearrangements[4] and in transacylation reactions.[5] This approach is particularly attractive for generating an antibody that catalyzes a Diels–Alder reaction since this reaction proceeds through a highly ordered transition state for which $\Delta S^{\ddagger}$ is typically on the order of −30 to −40 entropy units.[6] Recently, Hilvert and coworkers reported that antibodies generated against a stable bicyclic adduct resembling the product of the addition of a cyclic diene and dienophile catalyzed a Diels–Alder reaction.[7] Tetrachlorothiophene 1,1-dioxide was used as the diene since extrusion of sulfur dioxide from the Diels–Alder adduct minimizes product inhibition.

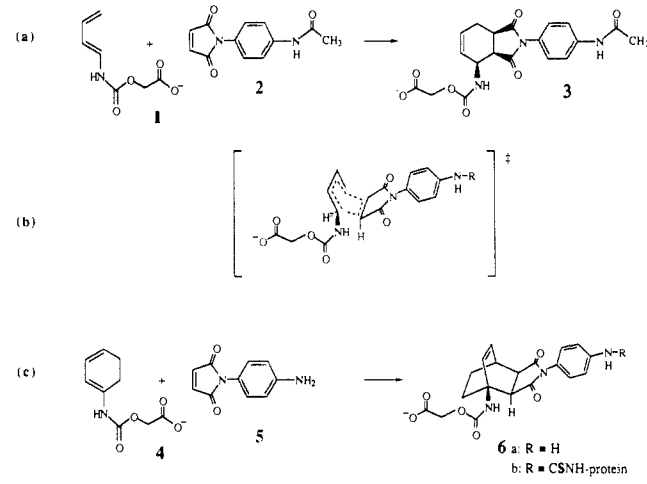

Figure 1. (a) Antibody-catalyzed Diels–Alder reaction. (b) Schematic representation of the transition state. (c) Synthesis of the transition-state analogue **6**.

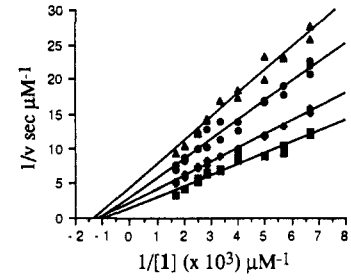

Figure 2. Lineweaver–Burk plot with the dienophile held at nine fixed concentrations while the diene was increased through nine distinct concentrations from 150 to 600 μM. All runs were performed in duplicate; only four lines are shown here for clarity (▲ = 150 μM 2, ● = 250 μM 2, ◆ = 350 μM 2, ■ = 600 μM 2. An analogous plot was also constructed with the diene held at fixed concentrations.

Our approach toward the design of a transition-state analogue (Figure 1) involves incorporation of an ethano bridge, which locks the cyclohexene ring of hapten **6** in a conformation that resembles the proposed pericyclic transition state[8] for the Diels–Alder reaction of cisoid diene **1** with dienophile **2**. As this geometry corresponds to a higher energy boat conformation of the product, it was anticipated that product inhibition would be minimized. Moreover, this design strategy is broadly applicable to Diels–Alder reactions involving acyclic dienes. We now report that antibodies generated to the transition-state analogue **6** catalyze the addition of the acyclic water-soluble diene **1** to the maleimide derivative **2** to give the cyclohexene product **3**.

Dienes **1** and **4** were prepared from the appropriate dienoic acid via formation of the acyl azide followed by a Curtius rear-

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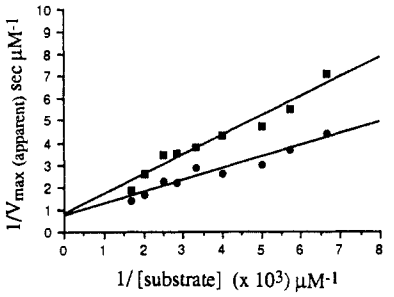

Figure 3. Replot of the apparent V_{max} values obtained from the Lineweaver-Burk plots versus concentration to provide the true V_{max} value as $1/(y$ intercept) and K_m values as $-1/(x$ intercept) (■ = fixed concentrations of diene; ● = fixed concentrations of dienophile).

rangement at 110 °C and subsequent trapping with methyl glycolate.[9] Only a single structural isomer was isolated from the Diels-Alder reaction with each of these dienes, and the endo conformation was assigned in both cases on the basis of nuclear Overhauser effect (NOE) cross peaks determined by 2D NOESY spectroscopy.[10] The isothiocyanate derivative of **6** was coupled to keyhole limpet hemocyanin (KLH), and the resulting protein conjugate **6b** was used to immunize Balb-c mice following a standard protocol.[11]

The kinetics of the Diels-Alder reaction of **1** with **2** were determined in 20 mM HEPES, 100 mM NaCl, pH 7.5 at 25 °C, both by HPLC[12] and by monitoring the change in absorbance at 247 nm.[13] One of 10 antibodies specific for hapten **6** (antibody 39, A11) demonstrated a large rate enhancement relative to the background reaction rate. The kinetics for this antibody-catalyzed reaction were determined by measuring the difference in initial rates between the catalyzed and background reactions. Catalysis of the Diels-Alder reaction by antibody 39, A11 was examined as a random, rapid equilibrium system, and Lineweaver-Burk plots were constructed by holding one substrate at a fixed concentration while varying the concentration of the second (Figure 2).[14] The apparent V_{max} values thus obtained were then plotted as a function of substrate concentration to give the true V_{max} as 1.35 μM s^{-1} and a k_{cat} value of 0.67 s^{-1} per binding site (Figure 3). The K_m values for diene **1** and dienophile **2** were determined to be 1130 and 740 μM, respectively. The apparent second-order rate constant, k_{cat}/K_m, is equal to 900 M^{-1} s^{-1} for the dienophile and 583 M^{-1} s^{-1} for the diene. The second-order rate constant for the background reaction, k_{uncat}, was found to be 1.9 M^{-1} s^{-1} in the reaction buffer at 25 °C and 0.002 M^{-1} s^{-1} in acetonitrile at 25 °C by using an integrated second-order rate equation and curve fitting with a nonlinear regression program.[15]

The dissociation constant of antibody 39, A11 for hapten **6** was measured by fluorescence quenching, and a K_D of 126 nM was determined by a Scatchard analysis.[16] The binding constant for the reaction product **3** was determined to be 10 μM by a similar analysis, indicating that while the product is bound approximately 100-fold less tightly than the hapten, the antibody binds the product **3** roughly 75 times tighter than either diene **1** or dienophile **2**. Currently we are investigating the mechanism of this antibody-catalyzed reaction with respect to stereochemistry, activation parameters, and the concerted nature of bond formation.

Acknowledgment. This work was supported by the Director, Office of Energy Research, Division of Material Sciences and Division of Energy Biosciences, Office of General Life Sciences, Structural Biology Division, of the U.S. Department of Energy under Contract No. DE-AC03-76 SF00098 and the Lucille Markey Charitable Trust. We thank Denton Hoyer for his preliminary work on this project, Bob Simpson for his assistance with the NOESY spectra, and Jim Prudent for his assistance with antibody production.

Supplementary Material Available: Full experimental details for the synthesis of **3** and **6** as well as the experimental details for the kinetic measurements (14 pages). Ordering information is given on any current masthead page.

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(12) HPLC assays were carried out with a Microsorb C8 reverse-phase column with a gradient starting at 10% acetonitrile in 50 mM NaOAc at pH 5.0 and increasing to 40% acetonitrile over 6 min. Product formation was quantitated against the internal standard, 2,4-dinitrobenzoic acid. The retention time of the product formed in the catalyzed reaction is identical with that of the endo product.

(13) Reaction velocities were determined by measuring the difference in initial rates between the catalyzed and background reactions. All reactions were carried out at 25 °C in 20 mM HEPES, 100 mM NaCl at pH 7.5 with 1% acetonitrile, and all catalyzed reactions were run with 1 μM antibody 39, A11. Since the product and the dienophile have identical extinction coefficients at 247 nm (ϵ_{247} = 17 000), the loss in absorbance reflects the reaction of the diene (ϵ_{247} = 28 000) and represents the rate of the catalyzed reaction. Velocities were determined from the slope of the linear portion of the absorbance change at less than 3% completion.

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New Approach to Boron-Stabilized Organometallics

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Boron-stabilized carbanions are versatile reagents that have found numerous synthetic applications.[1-3] They have been prepared via deprotonation of sterically hindered boranes[1] and stabilized alkyldialkoxyboranes[2] or via a boron-lithium exchange reaction.[3] We report herein a new general preparation of α-

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