Crossing Extreme Mechanistic Barriers by Antibody Catalysis: Syn Elimination to a Cis Olefin

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Received April 4, 1994

Among disfavored chemical transformations, the syn elimination reaction occupies a special place in organic chemistry.[1] In most systems, syn elimination through a stereoelectronically-defined eclipsed conformation must overcome formidable steric and torsional strain,[2] as well as orbital symmetry barriers.[3] Recently, antibody catalysis has been applied successfully to the execution of disfavored chemistry.[4] Here, we extend this work to the catalysis of the most difficult type of syn elimination reaction where an acyclic substrate is converted to a cis (*Z*) olefin.

For acyclic systems, it is generally accepted that antiperiplanar elimination is greatly favored over syn elimination.[5] Although the eclipsed syn coplanar transition state may be preferentially adopted over the staggered antiperiplanar transition state in constrained cyclic systems,[6] acyclic syn elimination is rare. Additionally, all accounts of acyclic syn elimination have been shown to provide a trans (*E*) olefin, resulting when the competing anti elimination suffers significant destabilizing steric interactions in route to the alternative cis olefin.[7] In fact, of the four possible elimination pathways (anti to trans, anti to cis, syn to trans, and syn to cis), syn elimination to a cis olefin is regarded as the least favored transformation of the group and, to our knowledge, has not yet been selectively achieved in an acyclic system.[1,8] Consistent with these generalizations, substrate **1** undergoes anti elimination to give exclusively the trans olefin **3** (Figure 1).[9] The design of hapten **5** was intended to elicit antibodies which would bind and lock substrate **1** in an eclipsed conformation, such that subsequent elimination would occur syn to afford selectively the cis olefin **4**. The bicyclo[2.2.1]heptane ring structure of hapten **5** ensured presentation of the phenyl and benzoyl substituents in the desired eclipsed arrangement (Figure 2), as opposed to a cyclohexyl framework which would have oriented these substituents in a gauche relationship. The primary amine of **5** was introduced in a position corresponding to the α-keto proton of substrate **1** to induce an amino acid side chain in the antibody binding pocket *capable of acting as a general base for the abstraction of this* proton.[10]

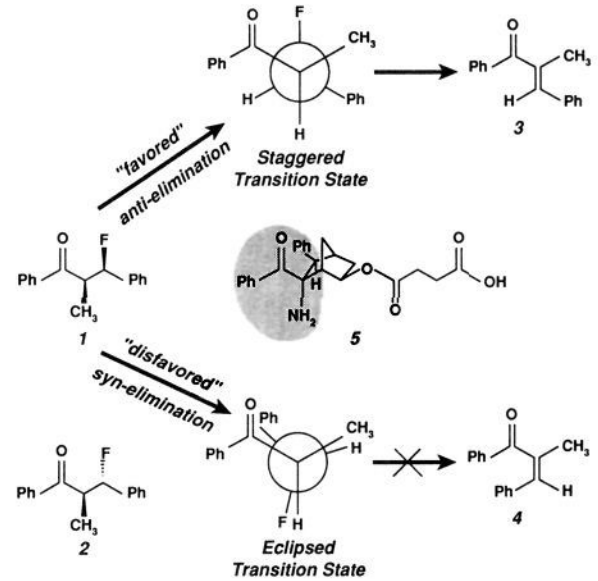

Figure 1. Mechanisms of syn (**1** to **4**) and anti (**1** to **3**) elimination. In the absence of antibody, only anti elimination is observed. The highlighted region of hapten **5** mimics the eclipsed syn coplanar transition state required for syn elimination of **1**.

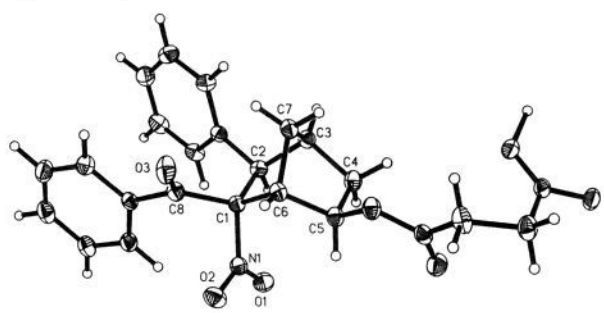

Figure 2. X-ray crystal structure of (1*R**,2*S**,4*R**,5*S**,6*S**)-6-benzoyl-6-nitro-5-phenylbicyclo[2.2.1]heptane 2-hemisuccinate. This compound was treated with H_2/Raney Ni to provide hapten **5**.

Compounds **1–5** were synthesized,[11] and the hapten **5** was conjugated to carrier protein keyhole limpet hemocyanin (KLH).[12] Immunization with the KLH conjugate and generation of monoclonal antibodies were performed as described previously.[13]

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(9) Reaction conditions: 15% aqueous DMSO, 100 mM CHES buffer, pH 9.0, 37 °C. No isomerization of either olefinic product (**3** or **4**) was observed under these conditions.
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(11) Synthesis of **5** will be reported elsewhere: Boger, D. L.; Lerner, R. A.; Cravatt, B. F. *J. Org. Chem.*, in press. Synthesis of **1** and **2**: 3-hydroxy-2-methyl-1,3-diphenyl-1-propanone was prepared (Brown, H. C.; Dhar, R. K.; Ganesan, K.; Singaram, B. *J. Org. Chem.* **1992**, *57*, 499–504) and subsequently treated with (diethylamino)sulfur trifluoride (DAST, 1.1 equiv) in CH_2Cl_2 at –78 °C for 1 h to afford a 4:1 mixture of **2** and **1**, respectively. Flash chromatography (1–4% Et_2O in hexanes) cleanly separated **1** and **2**, which were identified by correlation (NMR) with the corresponding bromides.[16] Treatment of either **1** or **2** with KOH (1 equiv) in MeOH afforded exclusively the trans product **3**. Compound **3** was then isomerized under UV light (5 h in benzene) to yield a 3:1 mixture of **4** and **3**, respectively. The authentic samples of **3** and **4** were cleanly separated by flash silica gel chromatography (1–2% Et_2O in hexanes).
(12) In a control experiment, **5** was shown to react exclusively with benzylamine under the following conditions: (1) EDCI (1.05 equiv), *N*-hydroxysuccinimide (1.5 equiv), DMF, 25 °C, 7 h; (2) benzylamine (1.5 equiv), room temperature, 10 h. No amine acylation of **5** was observed under these conditions.

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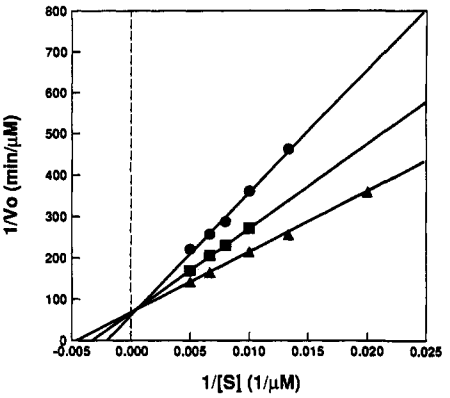

Figure 3. Lineweaver–Burk plot for antibody-catalyzed syn elimination (5 μM antibody 1D4, 15% aqeuous DMSO, 100 mM CHES buffer, pH 9.0, 37 °C). Rates were determined by analytical reverse-phase HPLC by measuring product formation relative to 4-methyl-3-nitroanisole as a standard: ▲, 0 μM inhibitor (hapten **5**); ■, 3 μM inhibitor; ●, 4.5 μM inhibitor. Antibody catalysis was completely inhibited by stoichiometric amounts of hapten **5**.

Twenty-six monoclonal antibodies specific for **5** were tested for catalysis. The rate of elimination of substrate **1** to **3** or **4** was assayed in the absence and presence of antibody at 37 °C in 15% aqueous DMSO, 100 mM CHES buffer, pH 9.0.[14] In the absence of antibody, only trans product **3** was generated with a first-order rate constant of 2.48×10^{-4} min^{-1}. One of the 26 antibodies, 1D4, was found to catalyze exclusively the syn elimination of substrate **1** to cis product **4**.[15]

The initial rate of syn elimination by 1D4, when measured as a function of substrate **1** concentrations, followed Michaelis–Menten kinetics (Figure 3). The kinetic constants K_m and k_{cat} were determined to be 212 μM and 2.95×10^{-3} min^{-1}, respectively. The catalytic activity of 1D4 was competitively inhibited by the addition of hapten **5**, indicating that catalysis occurs within the antibody binding pocket. The rate acceleration (k_{cat}/k_{uncat}) due to 1D4 catalysis could not be determined because in the absence of antibody, formation of cis product **4** was immeasurably slow under our reaction conditions. Catalysis of the syn elimination of substrate **1** in the absence of an observable k_{uncat} underscores the power of catalytic antibodies to accelerate energetically demanding reactions with high efficiency and selectivity.

Antibody 1D4 was also tested for its capacity to catalyze the anti elimination of substrate **2** to afford the cis olefin **4**.[16] The anti elimination of **2**, which would proceed through a staggered transition state to provide **4**, should be quite favored over the syn elimination of substrate **1**, which requires reaction through an eclipsed transition state to provide **4**.[1,17] Yet, 1D4 demonstrates the reverse selectivity in that it accelerates the syn elimination of **1** to **4** more efficiently than the anti elimination of **2** to **4**. This intriguing result emphasizes the fidelity of 1D4 for the eclipsed conformation of substrate **1**, in accord with the bicyclo[2.2.1]-heptane ring structure of the inducing hapten **5**. In essence, the binding energy of 1D4 has been directed toward recognition of the phenyl and benzoyl substituents of substrates **1** and **2** in an eclipsed orientation. Consequently, 1D4 appears more willing to permit the syn elimination of **1** from this conformation than the rearrangement of **2** to the staggered transition state required for the otherwise preferred anti elimination. Mechanistic investigations of 1D4 are underway to determine more precisely how the antibody performs the syn elimination reaction.

In assessing the future direction of catalytic antibodies, it becomes important to consider in what realms of chemistry they are capable of operating. More specifically, what limits may exist to the size of the energy barrier capable of being crossed by antibody catalysis? Preliminary estimates of the energy difference between the anti and syn elimination reactions of **1** to **3** and **4**, respectively, indicate an up to 5 kcal/mol separation,[18] likely making syn elimination of **1** to **4** the most energetically demanding reaction yet catalyzed by an antibody. Thus, the generation of an antibody capable of accelerating a highly disadvantaged syn elimination reaction has brought the level of "disfavored" chemistry amenable to antibody catalysis to a new extreme.

Acknowledgment. This work was financially assisted by the National Institutes of Health (GM-43858, K.D.J.) and an NSF predoctoral fellowship to B.F.C. We wish to thank Dr. Raj Chadha for performing the crystal structure analysis of (1*R**,2*S**,4*R**,5*S**,6*S**)-6-benzoyl-6-nitro-5-phenylbicyclo[2.2.1]-heptane 2-hemisuccinate.

Supplementary Material Available: Details of the X-ray structure determination for Figure 2 (16 pages); tables of observed and calculated structure factors (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(14) A solution of antibody in 100 mM CHES buffer, pH 9.0 at 37 °C, was treated with substrate **1** in DMSO to provide a final solution containing 5 μM antibody in 15% aqueous DMSO, 100 mM CHES, pH 9.0. All reaction rates were determined by analytical reverse phase HPLC (Vydac C_{18}, 41% acetonitrile in H_2O/0.1% trifluoroacetic acid) by measuring product formation relative to 4-methyl-3-nitroanisole as a standard.

(15) No detectable difference was observed between the rate of formation of the trans product **3** in the 1D4-catalyzed versus uncatalyzed reactions. In a control experiment, 1D4 showed no capacity to isomerize either olefinic product under the reaction conditions described.

(16) Interestingly, under the background conditions described previously, substrate **2**, like substrate **1**, eliminates entirely to the trans olefin **3**. No isomerization was witnessed for either product **3** or **4** under these conditions, and therefore, the conversion of **2** to **3** may be regarded as a syn elimination. In the absence of antibody, elimination of either substrate **1** or **2** to provide exclusively the trans olefin **3** is consistent with previous work[17] and may reflect primarily the distinct thermodynamic advantage **3** holds over its cis counterpart **4**.

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