Antibody-Catalysis of a Bimolecular Asymmetric 1,3-Dipolar Cycloaddition Reaction

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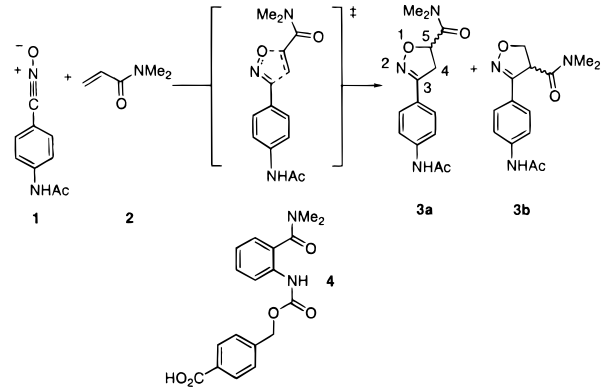
This contribution describes the first example of an antibody-catalyzed bimolecular [3 + 2] pericyclic process;[1] the 1,3-dipolar cycloaddition (1,3-DPC) reaction between the benzonitrile *N*-oxide **1** and *N*,*N*-dimethylacrylamide **2** to generate the 5-acylisoxazoline **3a** (Scheme 1).

The 1,3-DPC reaction is of tremendous synthetic utility, allowing rapid access into a wide diversity of chiral heterocyclic buiding blocks.[2] It is also a reaction for which there is no confirmed biological counterpart.[3] Frontier MO theory has been utilized to account for the regioselectivity observed in this reaction, which, due to favorable dipole LUMO-dipolarophile HOMO overlaps strongly favors formation of the 5-substituted isoxazoline.[4] The nature of the transition states of these reactions was one of this century's most contentious mechanistic issues,[5] but the asynchronous concerted mechanism is now accepted,[4b] with recent quantum mechanical studies providing high-level theoretical support for a planar, pericyclic transition state with aromatic character.[6] It was reasoned therefore that antibodies elicited against the planar aromatic core of hapten **4** may possess binding sites with the requisite low dielectric constant microenvironments, capable of both constraining the two reactants into a reactive conformation and reducing the translational entropy of the process.[7] In addition, the relative disposition of the *N*,*N*-dimethylamide and "flexible" benzyl carbamate substituents around the central planar core of hapten **4** would govern the orientation of the corresponding substrate substituents during the antibody-catalyzed reaction, potentially yielding regioselective catalysts for generation of either the favored 5- or disfavored 4-substituted products (**3a** or **3b** respectively).[8]

Scheme 1. 1,3-Dipolar Cycloaddition Reaction between Nitrile *N*-oxide **1** and Dipolarophile **2** Which Generates the Racemic 5- and 4-Acylisoxazoline Products **3a** and **3b**, Respectively[a]



[a] Hapten **4** was utilized as a transition state analog hapten for the generation of antibody catalysts.

The *N*-hydroxysuccinyl ester of hapten **4**[9] was coupled to carrier proteins and used to generate monoclonal antibodies via standard protocols.[10] Eighteen monoclonal antibodies specific for hapten **4** were produced, two of which catalyze the [3 + 2]-cycloaddition reaction between **1** and **2** in aqueous buffer.[11] Both antibodies catalyze completely regioselective formation of the 5-acylisoxazoline product **3a** and obey Michaelis−Menten kinetics. The most efficient catalytic antibody, 29G12, catalyzes the formation of **3a** with multiple turnovers (>50) and with no evidence of product inhibition.[12] 29G12 follows a completely random, sequential, *bi*−*uni* kinetic mechanism that proceeds via a ternary antibody−substrate complex (Figure 1A and 1B) and is stoichiometrically inhibited by hapten **4**.[13,14]

The kinetic parameters for the 29G12-catalyzed reaction [$k_{cat} = 0.34 \pm 0.01$ s^{-1}; $K_m(\mathbf{1}) = 3.4 \pm 0.4$ mM; $K_m(\mathbf{2}) = 5.8 \pm 0.4$ mM; lower estimate of the enhancement ratio $(k_{cat}/K_m(\mathbf{2})/k_{uncat},) = 4.3 \times 10^3$; effective molarity = 26 M; $k_{uncat} = 1.33 \pm 0.05 \times 10^{-2}$ M^{-1} sec^{-1} (measured at 4 °C)] compare very favorably with previous examples of antibody-catalyzed bimolecular cycloaddition processes.[1a−h] The proficiency of the 29G12-catalyzed

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(1) One bimolecular pericyclic reaction catalyzed by antibodies has been reported: the Diels−Alder reaction. For reviews, see: (a) Blackburn, G. M.; Datta, A.; Denham, H.; Wentworth, P., Jr. *Adv. Phys. Org. Chem.* **1998**, *31*, 249−392. (b) Wentworth, P., Jr.; Janda, K. D. *Curr. Opin. Chem. Biol.* **1998**, *2*, 138−144. (c) Hilvert D.; Hill, K. W. *Methods Enzymol.* **1991**, *203*, 352−369. For research articles, see: (d) Hilvert, D.; Hill, K. W.; Nared, K. D.; Auditor, M.-T. M. *J. Am. Chem. Soc.* **1989**, *111*, 9261−9262. (e) Braisted, A. C.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 7430−7431. (f) Gouverneur, V. E.; Houk, K. N.; De Pascual-Teresa, B.; Beno, B.; Janda, K. D.; Lerner, R. A. *Science* **1993**, *262*, 204−208. (g) Meekel, A. A. P.; Resmini, M.; Pandit, U. K. *J. Chem. Soc., Chem. Commun.* **1995**, 571−572. (h) Yli-Kauhaluoma, J. T.; Ashley, J. A.; Lo, C.-H.; Tucker, L.; Wolfe, M. M.; Janda, K. D. *J. Am. Chem. Soc.* **1995**, *117*, 7041−7047.

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(3) No naturally occurring biocatalyst for a 1,3-dipolar cycloaddition reaction has yet been either identified, isolated, or characterized. A recent report has outlined that Baker's yeast extract (*Saccharomyces cerevisiae*) can catalyze this reaction under certain conditions; (a) Rama Rao, K.; Nageswar, Y. V. D.; Sampathkumar, H. M. *J. Chem. Soc., Perkin Trans. 1* **1990**, 3199. (b) Rama Rao, K.; Nageswar, Y. V. D.; Bhanumathi, N.; Srinivasan, T. N. *Ind. J. Chem.* **1994**, *33B*, 171−172.

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(8) The reaction between **1** and **2** in the aqueous buffer system (see ref 11) yields the 5-substituted isoxazoline **3a** as a racemic mixture, exclusively. The activation energy calculated for the formation of the 5-substituted product **3a** (15.5 kcal mol^{-1}) is 4.9 kcal mol^{-1} lower than for formation of the 4-isomer in water (energies obtained with B3LYP/6-31+G* calculations).

(9) Hapten **4** was synthesized in three steps from 2-nitrobenzoic acid and will be reported elsewhere.

(10) (a) Harlow, E.; Lane, D. *Antibodies: A Laboratory Manual*; Cold Spring Harbor Lab: New York, 1988.

(11) Assays were performed in aqueous buffer [50 mM MES (pH 6.5), 50 mM NaCl, 4% v/v DMSO] at 4 °C unless otherwise stated. See Supporting Information.

(12) When programming antibody-catalysts for bimolecular associative processes the risk of product inhibition is a concern. The planar core of hapten **4** was considered to be sufficiently different from the isoxazoline ring of **3a** and **3b** such that product inhibition should be obviated.

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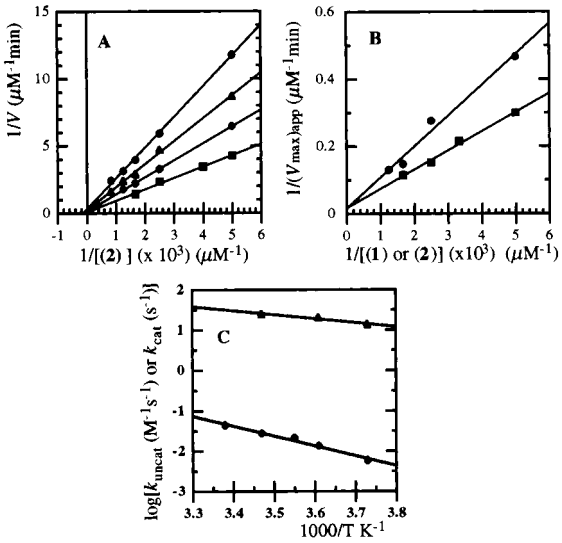

Figure 1. (A) Lineweaver−Burk plot of the initial velocities of cycloadduct **3a** formation in the presence of 29G12. Dipole **1** concentrations were fixed [(● 200 μM; $y = 2.282x + 0.300$, $r^2 = 0.999$), (▲ 280 μM; $y = 1.708x + 0.215$, $r^2 = 0.997$), (◆ 400 μM; $y = 1.257x + 0.152$, $r^2 = 1.000$) and (■ 600 μM; $y = 0.839x + 0.099$, $r^2 = 0.994$)], and the concentration of dipolarophile **2** was varied within the same range. All runs were performed in duplicate. (B) Replot of the V_{max}app obtained from the Lineweaver−Burk plots [(■ dipole; $y = 0.057x + 0.017$, $r^2 = 0.993$), (● dipolarophile; $y = 0.092x + 0.016$, $r^2 = 0.979$)] to obtain the true V_{max} value as 1/(y intercept) and Michalies-Menten constant (K_m) values as -1/(x intercept). (C) Arrhenius plot of the 29G12-catalyzed [▲; $y = -0.999x - 4.879$, $r^2 = 0.951$; mean temperature of determination (MTD = 278 K); A-parameter = 7.5×10^4 s^{-1}; E_a = 19.1 kjmol^{-1}] and noncatalyzed (◆; $y = -2.481x - 7.065$, $r^2 = 0.982$; MTD = 282 K, A-parameter = 11.6×10^6 M^{-1} s^{-1}; E_a = 47.5 kjmol^{-1}) 1,3-DPC reaction.

reaction [$k_{cat}/(K_m(\mathbf{1}) \times K_m(\mathbf{2}))/k_{uncat}$] is 1.5×10^6 M^{-1}, which is equivalent to a transition-state dissociation constant of 0.7 μM.

Catalytic asymmetric approaches to isoxazolines derived from nitrile *N*-oxides have been limited, in part, due to problems caused by the reactivity of this dipole with nitrogen and oxygen nucleophiles.[2b,15] However, 29G12 catalyzes this 1,3-DPC with good enantioselectivity, generating the 5-(*R*)-acylisoxazoline enantiomer **3a**[16a] in up to 98% ee,[16b] while concurrently binding the reactive dipole substrate **1** without covalent modification. This management of *N*-oxide substrates breaks new ground for antibody catalysts and the reactive intermediates they can utilize. Hapten **4** contains no chirality proximal to the loci of the stereogenic center in product **3a**. Therefore, the immune system, by nature of its inherent chirality, has elicited a stereochemical environment capable of exquisite stabilization of the enantiomeric transition state leading to *R*-**3a**.

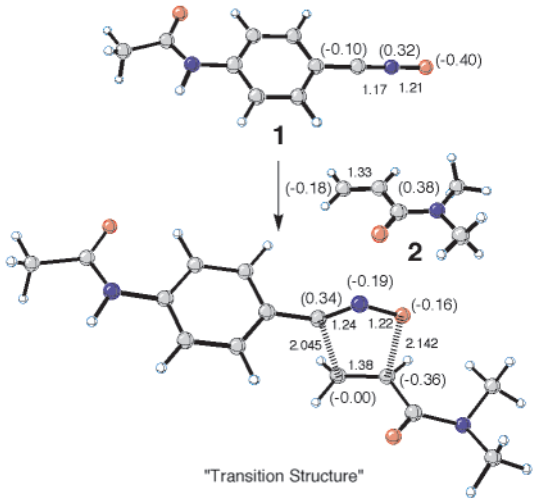

Figure 2. Computed structures of reactants **1** and **2** and transition structure (PM3). Bond lengths (Å) are given for atoms involved in the 1,3-dipolar cycloaddition. CHELPG charges (over atoms) are in au, computed with B3LYP/6-31+G*.

A comparison between the activation parameters for the noncatalyzed ($\Delta H^{\ddagger} = 10.8$ kcal/mol $\Delta S^{\ddagger} = -28.1$ eu) and antibody-catalyzed ($\Delta H^{\ddagger} = 4.0$ kcal/mol and $\Delta S^{\ddagger} = -38.1$ eu) reactions, reveals the remarkable phenomenon that overall, 29G12 stabilizes the free energy of the transition state for the 1,3-DPC by a significant reduction of the activation enthalpy (6.8 kcal/mol) rather than of the activation entropy (Figure 1C).[17] The binding-site of 29G12, programmed by the transition state mimic hapten **4** should reduce $\Delta S^{\ddagger}$, suggesting that additional features are contributing to the catalytic mechanism.

Modeling of the transition state for the reaction between **1** and **2**, reveals that the charges at the atoms involved in the cycloaddition change significantly during the reaction (Figure 2). Overall, the dipole moment of the transition state (4.5 D) is considerably less than the sum of the reactant dipole moments in the ground state (11.2 D). Furthermore, there is a striking contrast between the electrostatic potential of the nitrile *N*-oxide **1** and the CNO moiety in the transition state. Therefore, antibody-binding and stabilization of the lower-polarity transition state relative to ground state,[18] programmed by hapten **4**, coupled with the fortuitous potential for hydrogen-bonding at the amide carbonyl carbon of **2**[19] may well be assisting the 29G12 mechanism. These effects, both of which are enthalpic in nature, will destabilize the entropy of the process by necessitating solvent ordering and restriction of antibody binding-site residues respectively in the transition state. However, in the end, the composite character of these quantities and the probable role of solvation effects in determining them exclude drawing firm conclusions. In this regard X-ray crystallography is being utilized.

This contribution opens the door for the exploitation of asymmetric biocatalysis in a rich area of organic chemistry thought to be beyond the scope of protein catalysts and reaffirms the remarkable facility with which antibody catalysts can simultaneously control reactive intermediates and regio- and stereochemical reaction outcomes.

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Supporting Information Available: Synthetic information for **3a**, X-ray structural information of *R*-**3a**, details of kinetic assays (PDF). An X-ray crystallographic file (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) (a) *R*-**3a**: crystal system: orthorhombic; space group $P2_12_12_1$ (No. 19, D_2^4); unit cell parameters: $a = 6.7566$ (12) Å $\alpha = 90°$ $b = 9.1409$ (11) Å $\beta = 90°$ $c = 22.481$ (2) Å $\gamma = 90°$; temp. 296 K.; $Z = 4$. (b) The ee and absolute stereochemistry of the 29G12-catalyzed 1,3-DPC was determined by chiral HPLC and X-ray crystallography (ref 16a), respectively. See Supporting Information.

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