12. S. Sen, P. Brahma, S. K. Roy, D. K. Mukherjee, S. B. Roy, *Mol. Cryst. Liq. Cryst.* **100**, 327 (1983).
13. G. P. Wiederrecht, W. A. Svec, M. P. Niemczyk, M. R. Wasielewski, *J. Phys. Chem.* **99**, 8918 (1995).
14. M. R. Wasielewski, R. L. Smith, A. G. Kostka, *J. Am. Chem. Soc.* **102**, 6923 (1980).
15. J. C. Scott, L. Th. Pautmeier, W. E. Moerner, *J. Opt. Soc. Am. B* **9**, 2059 (1992).
16. H. J. Eichler, D. Gunter, D. W. Pohl, *Laser-Induced Dynamic Gratings* (Springer-Verlag, Berlin, 1986).
17. Q. Wang, R. M. Brubaker, D. D. Nolte, M. R. Melloch, *J. Opt. Soc. Am. B* **9**, 1626 (1992).
18. P. Y. Yan and I.-C. Khoo, *IEEE J. Quantum Electron.* **25**, 520 (1989).
19. The value for I_1 in this experiment is given by the relation

$$I_1 = I_0(1 - \eta_T) \quad (2)$$

where I_0 is the intensity in beam 1 after the sample in the absence of diffraction and η_T is the total diffraction efficiency of the grating. For the case of an applied electric field of 0.4 kV/cm, the value for I_1 was 68% (34 mW/cm^2) of the value of I_0 without the electric field. In other words, 32% of the total power lies in the diffracted spots. It is reasonable to consider the 34 mW/cm^2 as the correct value of I_1 for the beam coupling calculations. Subsequently, with both beams incident on the sample, beam 1 was found to increase in intensity to 64 mW/cm^2.
20. A. G. Chen and D. J. Brady, *Opt. Lett.* **17**, 441 (1992).
21. We gratefully acknowledge support from the Office of Computational and Technological Research, Division of Advanced Energy Projects, U.S. Department of Energy, under contract W-31-109-ENG-38.

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Efficient Aldolase Catalytic Antibodies That Use the Enamine Mechanism of Natural Enzymes

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Antibodies that catalyze the aldol reaction, a basic carbon-carbon bond-forming reaction, have been generated. The mechanism for antibody catalysis of this reaction mimics that used by natural class I aldolase enzymes. Immunization with a reactive compound covalently trapped a Lys residue in the binding pocket of the antibody by formation of a stable vinylogous amide. The reaction mechanism for the formation of the covalent antibody-hapten complex was recruited to catalyze the aldol reaction. The antibodies use the ε-amino group of Lys to form an enamine with ketone substrates and use this enamine as a nascent carbon nucleophile to attack the second substrate, an aldehyde, to form a new carbon-carbon bond. The antibodies control the diastereofacial selectivity of the reaction in both Cram-Felkin and anti–Cram-Felkin directions.

One of the major goals of organic chemistry is to use the understanding of reaction mechanisms to design new catalysts. This is often not easy because one must address intermediates that are of high energy and complex structure. Antibody catalysts offer an interesting solution to the problem in that they can be programmed by the experimenter to interact with the rate-limiting transition state of a chemical reaction to lower its energy and increase the reaction rate (*1*), but even here catalyst design is usually limited to the more global aspects of the transition state rather than the detailed reaction mechanism. Thus, while one can deal with high-energy charges, stereoelectronic, and geometrical features that appear along the reaction coordinate, the organization of multiple complex reaction intermediates remains difficult.

For some reactions, the problem of complex intermediates may be solved by using relatively reactive compounds rather than the more usual inert antigens to immunize animals or select antibodies from libraries such that the process of antibody induction involves an actual chemical reaction in the binding site (*2*). This same reaction then becomes part of the catalytic mechanism when the antibody interacts with a substrate that shares chemical reactivity with the antigen used to induce it. To test these ideas, we have studied the aldol condensation which is, arguably, the most basic C–C bond forming reaction in chemistry and biology. A variety of effective reagents have been developed to control the stereochemistry of the aldol, but these reagents are stoichiometric and require preformed enolates and extensive protecting-group chemistry (*3*). Recently, catalytic aldol reactions that use preformed enolates have been developed, including the Mukaiyama cross-coupling aldol (*4*). A number of enzymes catalyze the aldol condensation, and although much is understood about their mechanism (*5*) they accept a limited range of substrates (*6*). Thus, our goal was to induce antibodies that use the reaction mechanisms that give aldolases their efficiency but that take advantage of the range of substrates and stereochemical specificities available with antibodies.

Two mechanistic classes of aldolase enzymes have evolved (*7*). Class I aldolases utilize the ε-amino group of a Lys in the active site to form a Schiff base with one of the substrates, which activates the substrate as an aldol donor. Class II aldolases are metalloenzymes that facilitate enolate formation by coordination to the substrate's carbonyl oxygen. We chose class I aldolases as our model (Fig. 1A). The reaction is bimolecular and proceeds through covalent catalysis through multiple intermediates. An iminium ion or Schiff base forms that acts as an electron sink, which lowers the activation energy (E_a) for proton abstraction from the Cα atom and subsequent enamine formation. The enamine acts as the carbon nucleophile, or aldol donor, which reacts with an aldehyde electrophile, the aldol acceptor, to form a new C–C bond. The Schiff base is then hydrolyzed and the product is released (in this case, a β-hydroxy ketone). The essence of the mechanism is the formation of the enamine, which is the nascent carbon nucleophile. Although transition state models have been proposed for aldol reactions involving metals (*8*), models for the enamine case remain to be studied.

We designed haptens that could both trap the requisite Lys residue in the active site to then form the essential enamine intermediate and induce the appropriate binding sites for the two substrates to overcome the entropic barrier intrinsic to this bimolecular reaction. The simple 1,3-diketone hapten **1** provides elements of both a chemical and entropic trap (Fig. 1B). In water, the keto-form of the hapten shown predominates over the enol-form at a 3:1 ratio (*9*). The reaction coordinates of the aldol addition and the reaction mechanism expected when the hapten interacts with some antibodies share several common intermediates. In both cases, a tetrahedral carbinolamine intermediate forms that dehydrates to afford the cationic iminium that tautomerizes to the enamine. It was expected that antibodies induced according to the haptenic reaction mechanism would stabilize the analogous transition states and cationic intermediates along the reaction coordinate of the aldol reaction. The driving force for the reaction of the 1,3-diketone hapten with the antibody is the formation of a stable covalent vinylogous amide or conjugated enamine between the hapten and the ε-amino group of Lys. Calculations that make use of the Woodward rules for enones indicated that the vinylogous amide would have an absorption maximum in the ultraviolet (UV) that would allow for its identification, λ_{max} = 318 nm (*10*). The stability and spectral characteristics of this type of compound were previously noted in the studies of acetoacetate decarboxylase by Westheimer and co-workers (*11*). We expected an entropic advantage by incorporation of the second substrate (aldol acceptor) in the diketone chemical trap. Entropic effects could provide as much as 10^8 to 10^{11} to

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the rate acceleration of natural enzymes (*12*).

The reaction we studied is the aldol addition of acetone to 3-phenylpropionaldehyde derivatives. The second substrate is represented by the 3-phenylpropiononyl portion of the hapten. The tethering of the two substrates in the diketone hapten would present a substrate complex wherein the Heathcock angle (*13*, *14*) for attack of the enamine on the aldehyde would be distorted to the extreme of 90° in the rate-determining transition state of C–C bond formation. We did not expect this to be a major impediment in the catalytic reaction because rotation of both enamine and aldehyde faces should provide a reasonable Heathcock angle (Fig. 2).

The diketone hapten **1** was synthesized (Fig. 3) and coupled to keyhole limpet hemocyanin. After immunization of 129 G^{IX+} mice, 20 hybridomas producing antibodies to **1** were obtained with standard methods (*15*). Antibodies from each cell-line were purified by ammonium sulfate precipitation, anion exchange, and protein G affinity chromatography (*16*).

All 20 antibodies were screened in a microtiter plate assay for their ability to form the proposed stable vinylogous amide **2** by incubation of 20 μM antibody with 100 μM of the diketone hapten **1** (Fig. 4). Two antibodies, 38C2 and 33F12, demonstrated a strong absorption maximum at 316 nm characteristic of the proposed vinylogous amide **2**, approximating the calculated absorption maximum in the absence of protein of 318 nm (Fig. 4). Incubation of **1** with Lys under identical conditions resulted in no increase in absorbance. The extinction coefficient of the antibody-enamine complex was determined to be 15,000 cm^{-1} M^{-1} after subtraction of the absorbance of the antibody (Fig. 5A), approximating that observed in the reaction of acetopyruvate with the enzyme acetoacetate decarboxylase, 19,000 cm^{-1} M^{-1} (*11*).

Because the antibodies are expected to form an enamine with acetone in the synthetic reaction, observation of the vinylogous amide chromophore should not be dependent on the aldol acceptor (benzyl) portion of the hapten. We tested acetylacetone as the minimal diketone expected to generate the chromophore. Both antibodies reacted with this compound and produced the expected absorbance spectrum. To determine the stoichiometry of the antibody-enamine complex, a titration of the antibody with acetylacetone was carried out (*11*). If we assume that the reaction that forms the enamine is irreversible, the stoichiometry of the titration should correspond to the concentration of antibody active sites. The titration gives a ratio of acetylacetone to antibody of 1.9, indicating that each of the two identical antigen binding sites of the antibody form the vinylogous amide adduct (Fig. 5B). Catalysis of the formation of the vinylogous amide was essentially complete upon mixing of the antibody with hapten and sufficiently rapid that determination of the rate of this reaction would require stopped-flow kinetic studies.

Antibodies 38C2 and 33F12 were assayed for their ability to catalyze the addition of acetone to aldehyde **3** (Table 1). Consumption of **3** and production of the β-hydroxy ketone **4** were monitored by reversed-phase high-performance liquid chromatography (HPLC) (*17*). Aldol addition catalyzed by both antibodies followed Michaelis-Menten kinetics. Generation of acetone and aldehyde **3** from the β-hydroxy ketone **4** in the retro-aldol reaction by the antibodies was monitored by following the decrease in UV absorbance at 340 nm in a coupled enzymatic assay with alcohol dehydrogenase and β-nicotinamide adenine dinucleotide, reduced form (NADH) (*18*). Production of aldehyde **3** (*19*) was monitored by its subsequent reduction by alcohol dehydrogenase and consumption of NADH. The retro-aldol reaction was also studied by HPLC and the same results were obtained (*17*). The Michaelis constant K_M and catalytic rate constant k_{cat} values were 54 μM and 4.4 × 10^{-3} min^{-1}, respectively, for antibody 38C2. The remaining 18 antibodies were unable to catalyze the synthetic and retrosynthetic aldol reactions, indicating that only those that formed the critical intermediate were active.

We studied the ability of the hapten **1** and acetylacetone to inhibit the aldol reaction. When three equivalents of either hapten **1** or acetylacetone were provided prior to the aldol addition or retro-aldol assays, catalytic activity was completely inhibited,

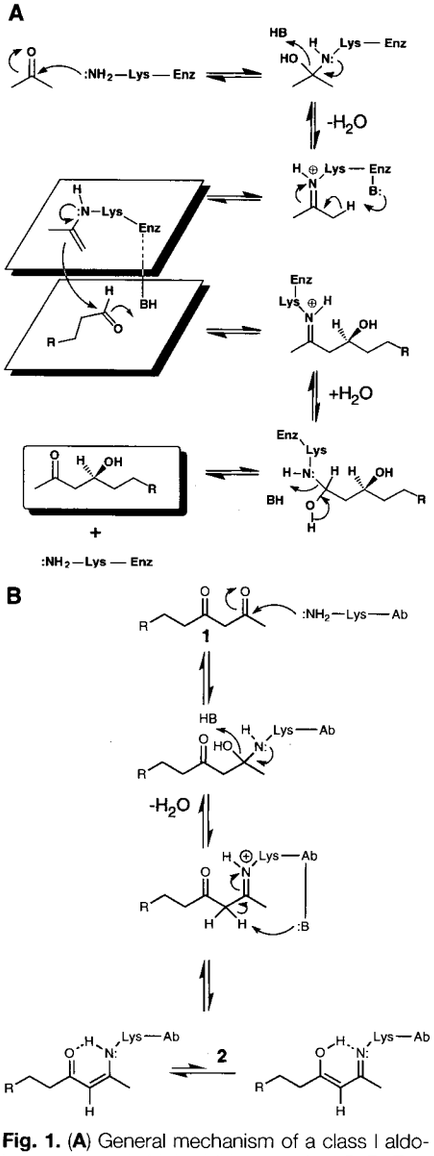


Fig. 1. (**A**) General mechanism of a class I aldolase–catalyzed aldol-addition reaction (*5*, *7*) (Enz, enzyme; B, base). (**B**) Mechanism of trapping the essential ε-amino group of a Lys residue in the antibody (Ab) binding pocket by using the 1,3-diketone hapten **1**. The formation of the stable covalent vinylogous amide **2** can be detected at λ = 316 nm (ε = 15,000). R = *p*(HOOC$(CH_2)_3$-CONH)C_6H_4–.

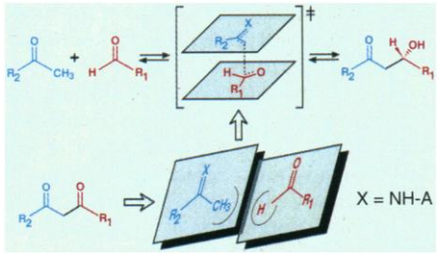


Fig. 2. The 1,3-diketone hapten structure contains the elements of a chemical and entropic trap. The binding pocket induced with the hapten **1** does not preclude attainment of a reasonable Heathcock angle for attack of the aldol donor on the acceptor. A proper attack geometry is attained by simple rotation of both enamine and aldehyde faces.

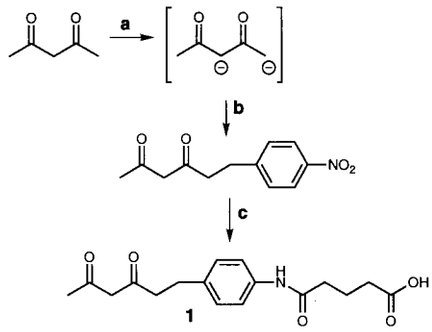


Fig. 3. Synthesis of hapten **1**. (a) LDA [2 equivalents (eq)], THF, 40°C, 1 hour. (b) 4-nitrobenzylbromide, hexamethylphosphoramide, −78° to −40°C, 48% yield. (c) (i) Pd/C, H_2, ethanol; (ii) glutaric anhydride, CH_2Cl_2, 74% yield.

showing that trapping of the enamine intermediate with the 1,3-diketones precludes catalysis involving the Lys ε-amino group. To establish that enamine formation with the hapten in the trapping assay and acetone in the catalytic assay are dependent on the same Lys residue, antibodies incubated with acetone were treated with $NaBH_4$. Reduction with $NaBH_4$ of the imine intermediate formed in the reaction of acetone with the Lys ε-amino group in the antibodies would result in irreversible isopropylation of the essential amine (5). After treatment, the antibodies could not form the vinylogous amide with the diketones. These experiments provide evidence that the reaction mechanism and residues induced with the 1,3-diketone hapten are the same as those recruited in the catalytic reactions. The antibody aldolases showed a broad pH optimum between 6 and 10 approximating that observed with natural class I aldolases (5, 7). Thus, antibody aldolases recapitulate the mechanisms of natural class I aldolases in the catalysis of this complex, multistep reaction (Fig. 1A).

We probed the substrate specificity of these catalysts because the most limiting aspect of the application of natural enzymes in synthesis is their rather poor acceptance of a range of substrates (6). Although natural enzymes display broad specificity with respect to the aldol acceptor, the aldol donor is usually limited to the natural substrate. For example, among the ketones studied for antibody catalysis (Table 1), only acetone is a substrate for a natural enzyme. In contrast, antibody aldolases can use various aldol donors and acceptors. The antibodies accept acetone, fluoroacetone, chloroacetone, 2-butanone, 3-pentanone, 2-pentanone, and dihydroxyacetone as aldol donor substrates. In reactions with 2-butanone and 2-pentanone, the antibodies exhibit some control of the regioselectivity of the aldol addition by preferential formation of the most substituted enamine. The relative efficiency of catalysis with these substrates decreases 42-fold as reflected by k_{cat}/K_M in the acetone to pentanone series (Table 1, entries 1 and 3 to 5). The failure of the antibodies to accept acetaldehyde as a donor shows that the aldol addition is directed with a ketone as the aldol donor (Table 1, entry 6). In principle, the diketone hapten should induce antibodies that react at either of the two keto positions of the hapten **1**, thereby generating catalysts that direct the aldol addition in either direction (Table 1, entry 1 or 7). Antibodies 38C2 and 33F12 are restricted to direct the aldol addition with acetone or aliphatic ketones as donors and 3-phenylpro-

Table 1. Substrate specificity of antibody 38C2; R = $CH_3CONHC_6H_4CH_2$–. The kinetic parameters k_{cat} and K_m of each reaction were calculated with respect to the aldehyde. The aldol donors (acetone, 2-butanone, 3-pentanone, 2-pentanone, and acetaldehyde) were fixed at a constant concentration of 5% v/v in each experiment (*17, 19*). Products **7/8** and **9/10** were formed at ratios 94 to 4 and 73 to 27, respectively.

Reaction	k_{cat} (10^{-3} min^{-1})	K_M (μM)
3 → **4**	6.7	17
5 → **6**	83*	125*
	40	48
→ **7** + **8**	18	52
→	5.1	115
→ **9** + **10**	3.5	374
→ No reaction		
→ No reaction		

*Reaction using antibody 33F12.

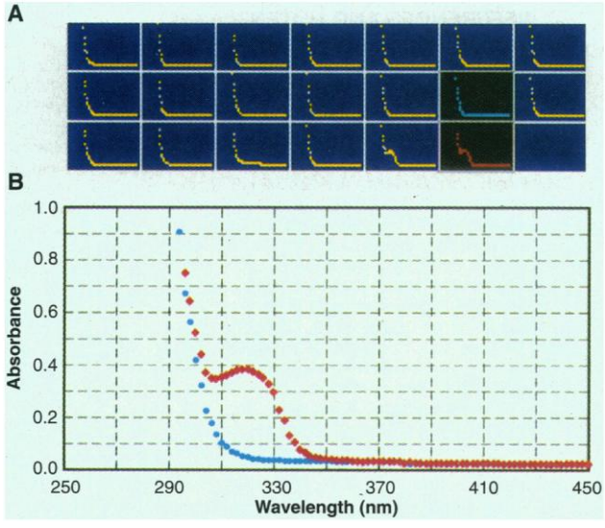


Fig. 4. (**A**) Screening of antibodies for the formation of the vinylogous amide intermediate **2**. Hapten 1, 5 eq, was added to 20 μM solutions of each antibody in PBS buffer (pH 7.5) in a microtiter plate format. (**B**) Antibodies with aldolase catalytic activity presented the typical absorption maximum of the vinylogous amide at 316 nm (example shown in red), whereas none of the inactive antibodies did (example shown in blue). Two antibodies out of 20 formed the intermediate **2** and were subsequently determined to be catalytic.

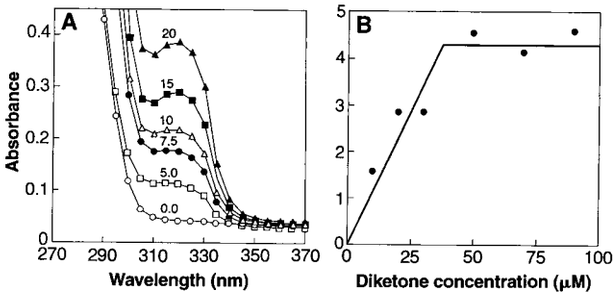


Fig. 5. (**A**) Determination of the extinction coefficient of **2**. A fixed concentration (100 μM) of hapten **1** was added to the indicated concentrations (micromolar) of 33F12. The antibody enamine complex could easily be detected at an antibody concentration as low as 2 μM. (**B**) The active sites of 38C2 were titrated with acetylacetone. The Ab concentration (20 μM) was fixed. Acetylacetone (0 to 4.5 eq) was added and the absorption measured at 316 nm. The intersection of the two lines corresponds to a ratio of 1.9 of acetylacetone to antibody 38C2, indicating that both binding sites of the antibody form the enamine adduct **2**.

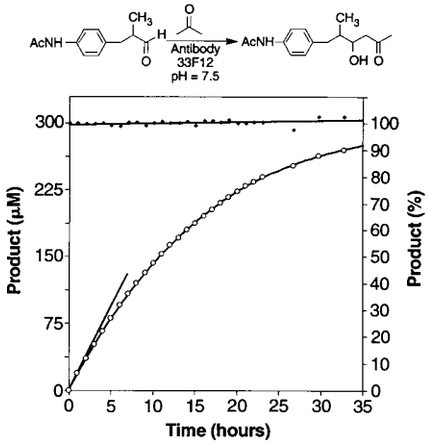
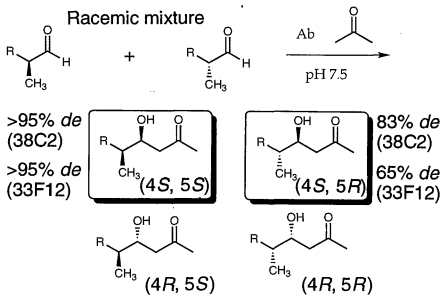


Fig. 6. The aldol addition reaction of aldehyde **5** and acetone was monitored over a 36-hour period in the presence of 1.5% catalyst (*20*) (Ac, acyl). The catalyst showed multiple turnovers (~2 turnovers per hour) and virtually no product inhibition. A 90% conversion could be obtained in the presence of excess acetone (5% v/v) to minimize the retro-aldol reaction. The perfect mass balance (top line) indicates that no side reactions, such as elimination or polymerization, occurred over that period. Thus, the antibody-catalyzed aldol reaction is an exceptionally mild method of C–C bond formation.

pionaldehyde derivatives as acceptors.

The reaction of the branched 3-phenylpropionaldehyde acceptor (Table 1, entry 2) with acetone was the most efficient and showed little product inhibition (Fig. 6) (*20*). In fact, less than 1 mole % catalyst is sufficient to achieve high conversion of substrate in a relatively short time. The reaction produces only the desired aldol product as each mole of aldehyde consumed is converted to the β-hydroxy ketone product (Fig. 6). For this reaction, the rate of the uncatalyzed background reaction at pH 7.5 under identical conditions used in the antibody assays has been determined, $k_{uncat} = 2.28 \times 10^{-7}\ M^{-1}\ min^{-1}$ (*21*). For 38C2 and 33F12, $(k_{cat}/K_M)/k_{uncat}$ is $\sim 10^9$. The efficiency of catalysis is due in a large part to an entropic advantage in the antibody-catalyzed reaction, which is reflected as a high effective molarity, $k_{cat}/k_{uncat} > 10^5$ M. The catalytic efficiency (k_{cat}/K_M) of antibody aldolases is only ~4000-fold lower than that of the most studied enzyme, fructose-1,6-bisphosphate aldolase (*5*). The catalytic efficiency of antibody 38C2 for the reaction given in entry 2, Table 1, is 64-fold greater than that obtained with catalysis by the enzyme 2-deoxyribose-5-phosphate aldolase (*22*).

We studied the product distribution of the antibody-catalyzed reaction of **5** with acetone after 30% conversion with a normal-phase HPLC chiral support column (Scheme 1) (*22*). Both antibodies catalyze



Scheme 1.

the diastereoselective addition of acetone to the *re*-face of **5** regardless of the stereochemistry at C-2 of this substrate. The aldol reactions follow the Cram-Felkin (*23*) mode of attack on (*S*)-**5** to generate the (4*S*,5*S*)-**6** product and the anti–Cram-Felkin mode of attack on (*R*)-**5** to generate the (4*S*,5*R*)-**6** product. The products formed with similar yields, so there was no kinetic resolution of the racemic aldehyde. The two antibodies differ in their control of the diastereofacial selectivity of the reaction and reflects the ability of the catalysts to orient **5** in the binding pocket of the antibody relative to the nucleophilic antibody-enamine of acetone. This differential binding is also reflected in differences in K_M for **5** in the antibodies (Table 1). The degree of stereochemical control of the reaction is exceptional as no stereochemical information was introduced into the hapten. The chemical reaction of the lithium enolate of acetone with (*S*)-2-methyl-3-phenylpropionaldehyde yields the analogous (4*S*,5*S*) product with a diastereomeric excess of 5% in favor of this Cram-Felkin product (*13*). The generation of the (4*S*,5*R*) and (4*R*,5*R*) products at an 11:1 ratio under antibody 38C2 catalysis shows a reversal of the typical Cram-Felkin stereoselectivity of the aldol, in this case following the disfavored and energetically more demanding anti–Cram-Felkin mode of attack.

Catalysts that proceed by defined reaction mechanisms can be induced by immunization with reactive compounds. This approach is not limited to Schiff base or enamine mechanisms and may be used whenever the chemistry to be accomplished is beyond that easily achieved by even a concert of noncovalent interactions. These results provide further evidence of the utility of antibody catalysts to perform disfavored chemical transformations (*16*, *24*).

REFERENCES AND NOTES

1. R. A. Lerner, S. J. Benkovic, P. G. Schultz, *Science* **252**, 659 (1991).
2. C. F. Barbas III, A. S. Kang, R. A. Lerner, S. J. Benkovic, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7978 (1991); K. D. Janda *et al.*, *ibid.* **91**, 2532 (1994); P. Wirsching *et al.*, *Science*, in press.
3. C. H. Heathcock, *Aldrichim. Acta* **26**, 99 (1990); C. H. Heathcock, *Science* **214**, 395 (1981); D. A. Evans, *ibid.* **240**, 420 (1988); S. Masamune, W. Choy, J. Peterson, L. R. Sita, *Angew. Chem. Int. Ed. Engl.* **24**, 1 (1985); D. A. Evans, J. V. Nelson, T. R. Taber, *Top. Stereochem.* **13**, 1 (1982); C. H. Heathcock *et al.*, in *Comprehensive Organic Synthesis*, B. M. Trost, Ed. (Pergamon, Oxford, 1991), vol. 2, pp. 133–319; I. Paterson, *Pure Appl. Chem.* **64**, 1821 (1992).
4. S. Kobayashi, H. Uchiro, I. Shiina, T. Mukaiyama, *Tetrahedron* **49**, 1761 (1993); K. Furuta, T. Maruyama, H. Yamamoto, *J. Am. Chem. Soc.* **113**, 1041 (1991); T. Bach, *Angew. Chem. Int. Ed. Engl.* **33**, 417 (1994), and references therein; E. M. Carreira, W. Lee, R. A. Singer, *J. Am. Chem. Soc.* **117**, 3649 (1995).
5. C. Y. Lai, N. Nakai, D. Chang, *Science* **183**, 1204 (1974); A. J. Morris and D. R. Tolan, *Biochemistry* **33**, 12291 (1994).
6. C.-H. Wong and G. M. Whitesides, *Enzymes in Synthetic Organic Chemistry* (Pergamon, Oxford, 1994); M. D. Bednarski, in *Comprehensive Organic Synthesis*, B. M. Trost, Ed. (Pergamaon, Oxford, 1991), vol. 2, pp. 455–473; C. F. Barbas III, Y.-F. Wang, C.-H. Wong, *J. Am. Chem. Soc.* **112**, 2013 (1990); H. J. M. Gijsen and C.-H. Wong, *ibid.* **117**, 2947 (1995); C.-H. Wonget *et al.*, *ibid.*, p. 3333; L. Chen, D. P. Dumas, C.-H. Wong, *ibid.* **114**, 741 (1992).
7. W. J. Rutter, *Fed. Proc. Am. Soc. Exp. Biol.* **23**, 1248 (1964).
8. H. E. Zimmerman and M. D. Traxler, *J. Am. Chem. Soc.* **79**, 1920 (1957).
9. M. Moriyasu, A. Kato, Y. Hashimoto, *J. Chem. Soc. Perkin Trans. II* **1986**, 515 (1986).
10. E. Pretsch, J. Seibl, W. Simon, T. Clerc, *Tables of Spectral Data for Structure Determination of Organic Compounds* (Springer-Verlag, Berlin, ed. 2, 1989), p. U20.
11. W. Tagaki, P. Guthrie, F. H. Westheimer, *Biochemistry* **7**, 905 (1968).
12. M. I. Page and W. P. Jencks, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1678 (1971).
13. C. H. Heathcock and L. A. Flippin, *J. Am. Chem. Soc.* **105**, 1667 (1983).
14. E. P. Lodge and C. H. Heathcock, *ibid.* **109**, 3353 (1987).
15. G. Kohler and C. Milstein, *Nature* **256**, 495 (1975).
16. V. E. Gouverneur *et al.*, *Science* **262**, 204 (1993).
17. A RP-C18 column (MICROSORB-MV, 0.45 cm by 22 cm) was used with an isocratic program of 75/25; H_2O (0.1% trifluoroacetic acid)/H_2O:acetonitrile 1:1, at 1.5 ml/min, monitored at 254 nm. The retention times of the aldehyde **3** and the aldol product **4** are 6.35 and 8.78 min, respectively. For kinetic studies, the ketone concentration was fixed at 5% v/v and the concentration of **3** or **5** was varied from 30 to 200 μM in the study of the aldol addition reaction. Antibodies were also assayed after an additional purification step over an anion-exchange column with identical results.
18. A solution, 100 μl, pH 7.5, containing 0.2 mM EDTA, 100 mM tris, 0.045 mg of yeast alcohol dehydrogenase, 0.43 mg of NADH, and 4 mM β-hydroxy ketone **4** was introduced into four wells of a microtiter plate. Antibodies 38C2 and 33F12 (100 μl, 34.6 μM, tris buffer, pH 7.5) were added to two different wells; 100 μl buffer only was added to the remaining wells, which were the blanks. The UV absorbance was measured at 340 nm every 15 min for 24 hours. The absorbance of the blanks was subtracted from the catalyzed reactions, and the rate was determined by using $\varepsilon = 6220\ cm^{-1}\ M^{-1}$. Antibodies 38C2 and 33F12 had the same $k_{cat} = 4.53 \times 10^{-3}\ min^{-1}$, which correlates well with the HPLC measurements.
19. The aldehydes **3** and **5** were prepared by using the Heck reaction [T. Jeffrey, *J. Chem. Soc. Chem. Commun.* **1984**, 1287 (1984)]. The products of the aldol additions of entries 1 to 5 have been prepared by independent synthesis. Typically, 50 to 100 mg aldehyde, 1 ml ketone, 4 ml H_2O, and 10 μl of saturated NaOH solution were shaken for 1 hour. The products were separated and purified by preparative reversed-phase HPLC. All new compounds were characterized by ^1H nuclear magnetic resonance (NMR), ^{13}C-NMR, and infrared and mass spectrometry.
20. The reaction was monitored by HPLC as in (*17*) but at 77/23 ratio instead of 75/25. The retention times for the aldehyde **5** and the β-hydroxy ketone **6** are 19.2 and 21.92 min, respectively.
21. J.-L. Reymond and Y. Chen, *Tetrahedron Lett.* **36**, 2575 (1995); J.-L. Reymond, personal communication.
22. The four diastereoisomers have been separated on a DAICEL Chiralpak OJ column with an isocratic program 7/1; hexane/ethanol, 1 ml/min, 254 nm. The retention times for the four isomers were: 19.74 (4*R*,5*R*), 23.32 (4*R*,5*S*), 25.15 (4*S*,5*R*), and 27.91 min (4*S*,5*S*). The relative configuration had been determined previously [see (*20*)]. The absolute configuration was deduced from an experiment wherein the catalyst was 2-deoxyribose-5-phosphate aldolase (DERA). DERA forms exclusively the aldol product possessing the (*S*) configuration at C-4 [see (*6*)]. The aldol product generated by DERA consists of a 1:4.5 mixture of (4*S*,5*R*)-**6** (92% diasteriomeric excess, *de*) and (4*S*,5*S*)-**6** (>95% *de*). The kinetic parameters of this particular transformation were $k_{cat} = 4.5 \times 10^{-2}\ min^{-1}$ and $K_m = 3400$ μM.
23. D. J. Cram and F. A. Abd Elhafez, *J. Am. Chem. Soc.* **74**, 5828 (1952); M. Chérest, H. Felkin, N. Prudent, *Tetrahedron Lett.* **1968**, 2199 (1968); N. T. Ahn, *Top. Curr. Chem.* **88**, 145 (1980).
24. K. D. Janda, C. G. Shevlin, R. A. Lerner, *Science* **262**, 204 (1993); B. F. Cravatt, J. A. Ashley, K. D. Janda, D. L. Boger, R. A. Lerner, *J. Am. Chem. Soc.* **116**, 6013 (1994).

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