Aldolase Antibodies of Remarkable Scope

Torsten Hoffmann, Guofu Zhong, Benjamin List, Doron Shabat, James Anderson, Svetlana Gramatikova, Richard A. Lerner,* and Carlos F. Barbas III*

Contribution from The Skaggs Institute for Chemical Biology and the Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received October 23, 1997

Abstract: This paper describes the substrate specificity, synthetic scope, and efficiency of aldolase catalytic antibodies 38C2 and 33F12. These antibodies use the enamine mechanism common to the natural Class I aldolase enzymes. Substrates for these catalysts, 23 donors and 16 acceptors, have been identified. The aldol acceptor specificity is expected to be much broader than that defined here since all aldehydes tested, with the exception polyhydroxylated aldehydes, were substrates for the antibodies. 38C2 and 33F12 have been shown to catalyze intermolecular ketone−ketone, ketone−aldehyde, aldehyde−ketone, and aldehyde−aldehyde aldol addition reactions and in some cases to catalyze their subsequent dehydration to yield aldol condensation products. Substrates for intramolecular aldol reactions have also been defined. With acetone as the aldol donor substrate a new stereogenic center is formed by attack on the *si*-face of the aldehyde with ee's in most cases exceeding 95%. With hydroxyacetone as the donor substrate, attack occurs on the *re*-face, generating an α,*β*-dihydroxy ketone with two stereogenic centers of the α-syn configuration in 70 to >98% ee. With fluoroacetone donor reactions, the major product is a syn α-fluoro-*β*-hydroxy ketone with 95% ee. Studies of retroaldol reactions demonstrate that the antibodies provide up to 10^8-fold enhanced efficiency relative to simple amine-catalyzed reactions.

Introduction

The aldol reaction is arguably one of the most important C−C bond-forming reactions employed in synthetic transformations. Traditionally, the aldol reaction has been a proving ground for the development of asymmetric synthetic strategies. In the 1980s the aldol reaction experienced a renaissance with the development of numerous strategies to effect highly stereoselective aldols.[1] Generally, this has been most successfully achieved through the use of stoichiometric quantities of chiral auxiliaries. In recent years the design of stereoselective catalysts of the aldol reaction has become a topic of interest.[2] Most notable of these approaches is the Carreira aldol reaction where a chiral Ti(IV) complex (2−10 mol %) catalyzes the enantioselective addition of 2-methoxypropene to aldehydes with 66−98% ee.[2b] As a challenge to traditional organic methodology, the application of natural aldolase enzymes as synthetic catalysts has yielded numerous efficient syntheses of stereochemically complex molecules, particularly in the area of carbohydrate synthesis.[3] Since no asymmetric catalyst exhibits the scope of reactivity required to meet every synthetic challenge, there is a need for methodologies that allow for the development of asymmetric catalysts. This is true of both, transition metal based as well as enzyme-based catalysts. For example, while the Carreira Ti(IV) complex is limited in scope to the use of the enolate equivalent 2-methoxypropene, fructose 1,6-diphosphate aldolase is limited to the use of dihydroxyacetone phosphate as the aldol donor substrate.[3a]

To address the problem of the *de novo* generation of protein catalysts of the aldol reaction, we recently described the development of two aldolase catalytic antibodies 38C2 and 33F12.[4]

These antibodies were raised against the *β*-diketone hapten **1** which served as a chemical trap to imprint the lysine-dependent class I aldolase mechanism in the active site of the antibody. The suggested mechanism for the selection process of antibodies 38C2 and 33F12 during immunization is shown in Scheme 1. The *ϵ*-amino group of the lysine residue reacts with a carbonyl function of the *β*-diketone moiety of **1** to form a *β*-keto hemiaminal followed by dehydration to give a *β*-keto

(1) For reviews of the aldol reaction, see: (a) Heathcock, C. H. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1984; Vol. 3. (b) Evans, D. A.; Nelson, J. V.; Taber, T. R. *Top. Stereochem.* **1982**, *12*, 1. (c) Masamune, S.; Choy, W.; Peterson, J. S.; Sita, L. R. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 1. (d) Heathcock, C. H. *Aldrichim. Acta* **1990**, *23*, 99. Heathcock, C. H. *Science* **1981**, *214*, 395. Evans, D. A. *Ibid.* **1988**, *240*, 420. Masamune, S.; Choy, W.; Peterson, J.; Sita, L. R. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 1. Evans, D. A.; Nelson, J. V.; Taber, T. R. *Top. Stereochem.* **1982**, *13*, 1. Heathcock, C. H. et al. In *Comprehensive Organic Synthesis*; Trost, B. M., Ed.; Pergamon: Oxford, 1991; Vol. 2, pp 133−319. Peterson, I. *Pure Appl. Chem.* **1992**, *64*, 1821.

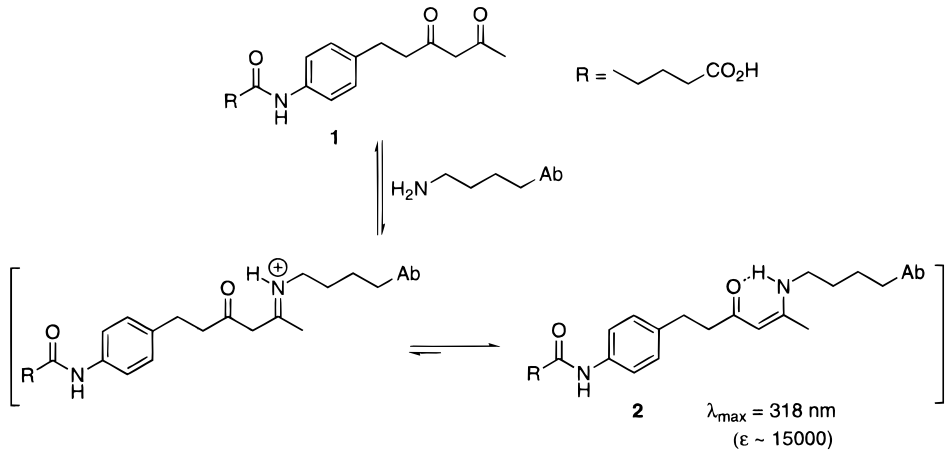
(2) (a) Yanagisawa, A.; Matsumoto, Y.; Nakashima, H.; Asakawa, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1997**, *119*, 9319 and references therein. Bach, T. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 417 and references therein. Yamada, Y. M. A.; Yoshikawa, N.; Sasai, H.; Shibasaki, M. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1871. (b) Carreira, E. M.; Lee, W.; Singer, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 3649.

(3) (a) Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443. (b) Wong, C.-H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 412−432. (b) Henderson, I.; Sharpless, K. B.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 558. (c) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994. Bednarski, M. D. In *Comprehensive Organic Synthesis*; Trost, B. M., Ed.; Pergamon: Oxford, 1991; Vol. 2, p 455. Gijsen, H. J. M., Wong, C.-H. *Ibid.* **1995**, *117*, 2947. Wong, C.-H. et al. *Ibid.* **1995**, *117*, 3333. Chen, L.; Dumas, D. P.; Wong, C.-H. *Ibid.* **1992**, *114*, 741.

(4) (a) Wagner, J.; Lerner, R. A.; Barbas III, C. F. *Science* **1995**, *270*, 1797. (b) Zhong, G.; Hoffmann, T.; Lerner, R. A.; Danishefsky, S.; Barbas III, C. F. *J. Am. Chem. Soc.* **1997**, *119*, 8131. (c) Barbas III, C. F.; Heine, A.; Zhong, G.; Hoffmann, T.; Gramatikova, S.; Björnestedt, R.; List, B.; Anderson, J.; Stura, E. A.; Wilson, E. A.; Lerner, R. A. *Science* **1997**, *278*, 2085.

S0002-7863(97)03676-7 CCC: $15.00
Published on Web 03/07/1998

Scheme 1. Mechanism of Trapping the Essential ϵ-Amino Group of a Lysine Residue in the Antibody Binding Site Using the β-Diketone Hapten **1**



imine that finally tautomerizes into a stable enaminone **2**. Consequently, the hapten is now covalently bound in the binding pocket. The mechanistic similarity between this stoichiometric reaction and the accepted enamine mechanism of class I aldolase enzymes has been discussed in detail elsewhere.[4] The formation of the enaminone has been monitored by UV spectroscopy (with hapten **1**: $\lambda_{max} = 318$ nm, $\epsilon \sim 15000$) and is complete within seconds to a few minutes, depending on whether antibodies were incubated with hapten **1**, or other diketones such as 2,4-pentanedione or 3-methyl 2,4-pentanedione. Antibodies 38C2 and 33F12 have been previously shown to catalyze aldol reactions of some aliphatic ketone donors with two different aldehyde acceptors having a 4-acetanilide substituent in the β-position as well as intramolecular aldol reactions that allowed for our recent antibody-catalyzed synthesis of the Wieland−Miescher ketone.[4b] Moreover, both antibodies were found to catalyze the decarboxylation reactions of aromatic β-keto acids by the formation of a Schiff base between the ϵ-amino group of the lysine residue and the keto group of the substrate.[5]

With antibodies 38C2 and 33F12 we have now addressed four issues: (i) scope and limitations of substrates for intermolecular crossed and self-aldols as well as intramolecular aldols, (ii) their stereoselectivity, (iii) kinetic parameters for these reactions to understand the nature of the binding pocket, and (iv) additional mechanistic studies to gain further insight into these catalysts.

Results and Discussion

Cross-Aldol Reactions. To define the scope of antibody catalysts 38C2 and 33F12 for the cross-aldol reaction we tested a wide variety of commercially available ketones as donors and a set of six different aldehydes (4-acetamidobenzaldehyde (**3**), 3-(4′-acetamidophenyl)propanal (**4**), 3-(4′-acetamidophenyl)-2-methylpropanal (**5**), 4-(4′-acetamidophenyl)butanal (**6**), 4-nitrobenzaldehyde (**7**), and 4-nitrocinnamaldehyde (**8**); Figure 1) as acceptors. To screen for donor activity, aldehyde **4** was chosen as the standard acceptor aldehyde since it bears a 4-acetamidophenyl group at C3. This portion of the molecule is closely related to the hapten structure **1** and may be specifically recognized by the antibodies. For ease of comparison we determined specific rates for these reactions under the following defined conditions: 1 M of donor, 500 μM of aldehyde **4**, and 0.4 mol % of antibody. As can be seen from

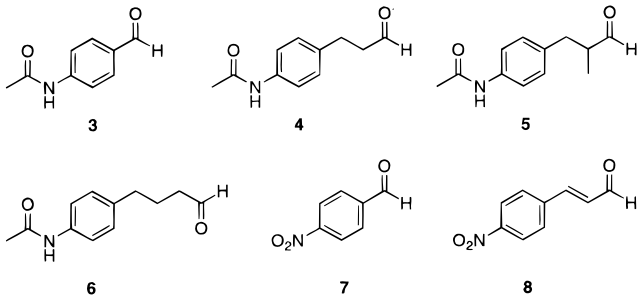


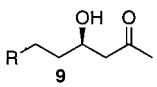
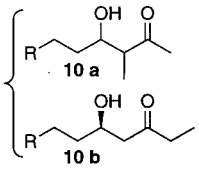
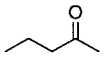
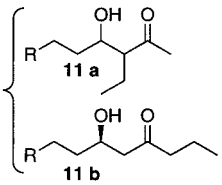
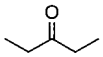
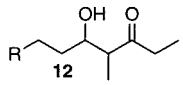
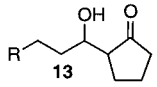
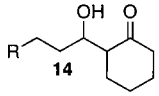
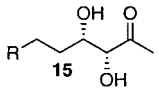
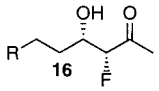
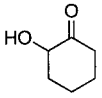
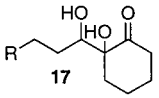
Figure 1. Aldehydes **3**−**8** as acceptors in the antibody-catalyzed cross-aldol reactions.

Table 1, a wide variety of different ketones undergo the aldol reaction with aldehyde **4** as proven by comparison of the retention times of the products using high performance liquid chromatography (HPLC) with independently chemically synthesized standards: aliphatic open chain (acetone, butanone, 2-pentanone, and 3-pentanone), aliphatic cyclic (cyclopentanone and cyclohexanone), functionalized open chain (hydroxyacetone and fluoroacetone), and functionalized cyclic (2-hydroxy cyclohexanone) ketones. A large variety of other ketones were also studied with aldehyde **4** with monitoring of the consumption of **4** and appearance of a new peak tentatively assigned as the β-hydroxy ketone product. Substrate ketones from all studies are summarized in Table 2. For structures of ketones that were tested and determined not to be substrates for these catalysts, see Supporting Information. The antibody-catalyzed aldol reactions were inhibited by addition of equimolar amounts of the hapten **1** or 2,4-pentanedione. Further control experiments were carried out using lysine or bovine serum albumin instead of the antibodies. No catalysis of the aldol reactions studied here was observed in these cases. A number of ketones, e.g. octanone, demonstrated limited solubility in aqueous medium perhaps precluding their availability to the catalysts. Further study of mixed solvent systems may allow conditions to be defined where substrates such as these are accepted by the catalyst. Of the ketones studied, reactions involving hydroxyacetone as the donor were the most efficient. The astonishingly high promiscuity of ab 38C2 for these physicochemically very different ketones is in remarkable contrast to the natural aldolases which tolerate minor, if any, change in the structure of the donor.[3]

Next, we focused on defining the specificity for acceptor aldehydes. We selected acetone, cyclopentanone, and hydroxyacetone as representative donors from the variety of ketones

(5) Björnestedt, R.; Zhong, G.; Lerner, R. A.; Barbas III, C. F. *J. Am. Chem. Soc.* **1996**, *118*, 11720.

Table 1. Donor Promiscuity: Specific Rates [μmol product $\times$ d^{-1} $\times$ μmol^{-1} ab] for Antibody-Catalyzed Cross-Aldol Reactions of a Variety of Ketones with Aldehyde **4** under the Following Defined Conditions: 1 M Donor, 500 μM **4**, and 0.4 Mol % Antibody (2 μM). R = 4-Acetamidophenyl

donor	product(s)	specific rate
	 **9**	2.5
	 **10 a** **10 b**	3.7
	 **11 a** **11 b**	0.4[a]
	 **12**	0.6[a]
	 **13**	6.4
	 **14**	nd[b]
	 **15**	54.3
	 **16**	5.5
	 **17**	nd[b]

[a] Specific rate was determined with 200 mM donor. [b] Not determined.

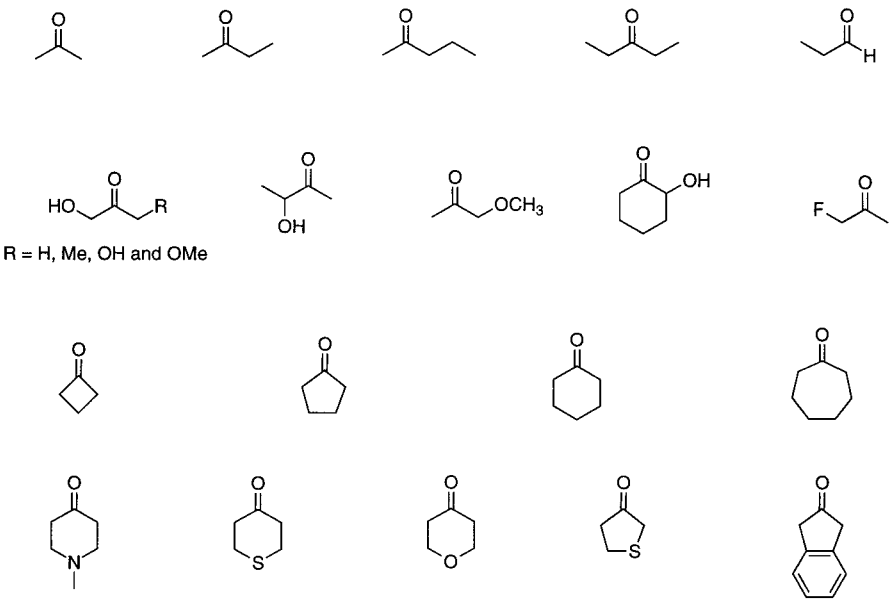
listed in Table 1 and tested these in the aldol reaction with aldehydes **3** and **5**−**8** (Figure 1). Again, identical conditions (as described above) were used for the determination of the corresponding specific rates (Table 3). All substrate combinations gave the expected products. Simple aliphatic aldehyde acceptors are described below (see Miscellaneous Aldol Reactions). A variety of polyhydroxylated acceptor aldehydes, glyceraldehyde, glucose, and ribose were also studied. These substrates were not accepted by these antibodies presumably due to the hydrophobic nature of the active site. These studies, together with those described below, suggest an acceptor specificity that is very broad, albeit limited to hydrophobic aldehydes.

To gain more detailed information about the catalytic system we chose some of the described reactions and studied initial rates, varying the substrate concentrations of either the donor or the acceptor while keeping the concentrations of the second reactant constant. All reactions with aldehydes **4**−**8** followed typical Michaelis−Menten kinetics by treating the data according to pseudo-first-order kinetics. Examples for antibodies 38C2 and 33F12 are provided in Supporting Information. Generally, no substrate or product inhibition was observed. Using 4-acetamidobenzaldehyde **3** at higher concentrations (2 mM and higher) substrate inhibition was found, presumably because of a reversible Schiff base formation between the active site lysine and the aldehyde. Michaelis−Menten kinetic data are summarized in the Supporting Information section. Typical values for the Michaelis constants K_M of the donors range from 1 mM to 1 M, reflecting the ability of the antibody to accept a wide variety of different ketones. Values for K_M of the acceptors **3**−**8** range from 10 to 500 μM. Obviously, the aromatic portion of these molecules is responsible for an increased recognition by the active site. Characteristic values for k_{cat} range from 10^{-3} to 1 min^{-1} and show a ratio of k_{cat}/k_{uncat} of 10^5 to 10^7 (see below for additional kinetic data). All data are reported per antibody active site where the antibody molecule has two active sites.

Self-Aldol Reactions. During the course of our investigations, we also addressed the question whether antibodies 38C2 and 33F12 are capable of catalyzing self-aldol reactions. Self-aldolization was observed with propionaldehyde, acetone, and cyclopentanone but not for compounds **4**−**6** (Table 4). Again, for ease of comparison, specific rates were determined using the following defined conditions: 0.1 M of ketone or aldehyde and 0.005 mol % of antibody. With these substrates the antibodies catalyzed the aldol condensation reaction which consists of two consecutive steps: the aldol addition and the subsequent elimination of water. Using propionaldehyde as substrate, the aldol addition product, 3-hydroxy-2-methylpentanal, was not detected as an intermediate but exclusively *trans*-2-methyl-2-pentenal **33** was detected, suggesting that the elimination of water is also catalyzed (vide infra). Compound **33** was obtained as single product and is not a substrate for a consecutive aldol addition reaction with a third propionaldehyde molecule. Interestingly, compound **33** acts as an acceptor if acetone is used as donor (see paragraph Miscellaneous Aldol Reactions). Acetone itself and cyclopentanone undergo the self-aldol condensation to give mesityl oxide **34** and compound **35**, respectively, if no aldehyde acceptor for a cross-aldol reaction is present which binds preferentially to the active site. These ketone self-aldol reactions are substantially slower, typically more than 500-fold slower, than the cross-aldol reactions involving these donors described above. In the cross-aldol reactions described above with acetone and cyclopentanone as donors, the acetone−acetone and cyclopentanone−cyclopentanone self-condensation products were not observed. Thus, self-aldolization activity in the cross-aldol reaction does not compromise isolation of the cross-aldol product. To elucidate the general mechanism of the self-aldol condensation reactions described above, we chose cyclopentanone as substrate and monitored the appearance of the aldol addition product **36** and its elimination product **35** by HPLC and gas chromatography (GC). In addition, ab 38C2 was incubated with independently synthesized intermediate **36**, and the appearance of **35** was monitored by HPLC. Indeed, both reaction steps are catalyzed with the aldol addition occurring faster than the elimination step. Kinetic study of the overall transformation of cyclopentanone to **35** revealed K_M = 845 μM, k_{cat} = 2 $\times$ 10^{-4} min^{-1}. The elimination reaction from **36** to **35** also followed Michaelis−

Table 2. Donor Substrates of Aldolase Antibodies 38C2 and 33F12

Aldol Donor Substrates



Menten kinetics (K_M = 750 μM, k_{cat} = 9 × 10^{-4} min^{-1} and k_{cat}/k_{uncat} = 2240). From these experiments we suggest a mechanism for the self-aldol condensation reaction of cyclopentanone as shown in Scheme 2 (pathway 1). The β-hydroxy iminium cation (formed in the active site after attack of the enamine of cyclopentanone at the carbonyl C-atom of a second molecule cyclopentanone) tautomerizes after loss of a proton into a β-hydroxy enamine which then loses water. This process is assisted by the electron donation from the enamine nitrogen atom.[6] The α,β-unsaturated iminium cation is finally hydrolyzed, and the aldol condensation product **35** is released while the lysine residue of ab 38C2 re-enters the catalytic cycle. An alternate mechanism in which the elimination step occurs in the background via free **36** is also shown in Scheme 2 (pathway 2) but seems less likely, since the elimination was shown to be catalyzed by ab 38C2 and inhibited by acetylacetone. No background reaction for the overall conversion of cyclopentanone to **35** in the absence of antibody could be observed following extended incubation for 2 months.

Intramolecular Aldol Condensations. To study intramolecular aldol reactions, 38C2 was incubated with three different aliphatic diketones: 2,4-hexanedione (**37**), 2,5-heptanedione (**39**), and 2,6-octanedione (**41**) (Scheme 3). No catalysis was observed in the reaction pathway from **37** to 3-methylcyclopent-2-enone **38**, presumably because of the Baldwin[7] disfavored *5-(enolendo-trig)* process involved in the attack of the enamine at C2 in substrate **37**. Although the corresponding ring closure (followed by water elimination) of **41** to give 3-methylcyclohept-2-enone **42** is Baldwin favored (a *7(enolendo-trig)* process) also in this case no product formation was observed. In contrast, the Baldwin favored ring closure reaction of 2,5-heptanedione (**39**) (a *6(enolendo-trig)* process) followed by elimination of water and giving 3-methylcyclohex-2-enone (**40**) was catalyzed by ab 38C2. Appearance of the product **40**—which is the sex pheromone of the Douglas Fir beetle[8]—was monitored by HPLC. The reaction followed Michaelis−Menten kinetics (Table 5) and showed an excellent rate acceleration. The antibody was also incubated with the corresponding intermediate, 3-hydroxy-3-methylcyclohexanone. As seen in the self-aldol condensation of cyclopentanone, the elimination step leading to **40** was also catalyzed by ab 38C2. In reported experiments,[4b] we tested the triketone **43** and the enantiomerically pure diketones (*S*)-**45** and (*R*)-**45** as substrates (Scheme 4). In all cases product formation (Wieland−Miescher ketone (*S*)-**44** and the ketones (*S*)-**46** and (*R*)-**46**), respectively) was catalyzed by 38C2 and proceeded with a rate which is comparable to the one found using the simple diketone **39** as substrate. All reactions followed Michaelis−Menten kinetics, the parameters of which are summarized in Table 5. We also examined 2-methyl-2-(3′-oxopentyl)-1,3-cyclohexanedione, 2-methyl-2-(3′-oxobutyl)-1,3-cyclopentanedione, and 2-methyl-2-(4′-oxopentyl)-1,3-cyclohexanedione as potential substrates for these antibodies; however, no reactions with these substrates were observed.

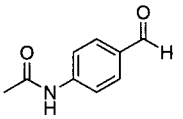
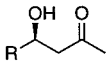
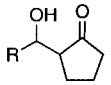
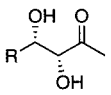
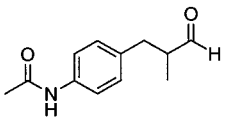
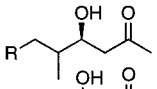
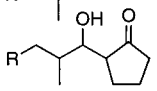
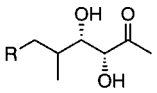
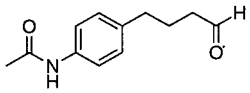
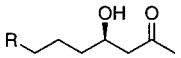
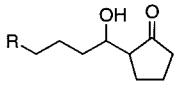
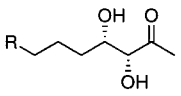
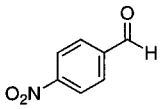
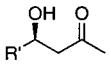
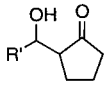
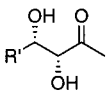
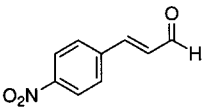
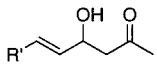
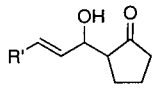
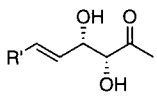
Miscellaneous Aldol Reactions. As described above, the self-aldol condensation of two molecules propionaldehyde terminates after the condensation step. The product, *trans*-2-methyl-2-pentenal (**33**) is not a substrate for a subsequent aldol addition (or condensation) of a third molecule propionaldehyde. In contrast, aldehyde **33** is a substrate if acetone is present as donor (Scheme 5). Surprisingly, the elimination of water from product **47** is not catalyzed by the antibody, although a thermodynamically favored $\alpha,\beta,\gamma,\delta$-unsaturated ketone could result. The binding pocket also accepts chain elongation in the acceptor structure as rationalized in 2,4-hexadienal (**48**). As seen with product **47**, the antibody does not catalyze the elimination of water from the aldol addition product **49** either. We also investigated whether propionaldehyde might be a donor for other aldehydes, which would bind preferentially to the binding pocket and would give a β-hydroxy aldehyde as product or, after elimination of water, the α,β-unsaturated aldehyde. To

(6) Hupe, D. J.; Kendall, M. C. R.; Spencer, T. A. *J. Am. Chem. Soc.* **1973**, *95*, 2271.

(7) Baldwin, J. E.; Lusch, M. J. *Tetrahedron* **1982**, *38*, 2939.

(8) Dickens, J. C.; Gutman, A.; Payne, T. L.; Ryker, L. C.; Rudinsky, J. A. *J. Chem. Ecol.* **1983**, *9*, 1383. Libbey, L. M.; Oehlschlager, A. C.; Ryker, L. C. *J. Chem. Ecol.* **1983**, *9*, 1533.

Table 3. Acceptor Promiscuity: Specific Rates [μmol product $\times$ d^{-1} $\times$ μmol^{-1} ab] for Antibody-Catalyzed Cross-Aldol Reactions of Acetone, Cyclopentanone, and Hydroxyacetone with Aldehydes **3** and **5**−**8** under the Following Defined Conditions: 1 M Donor, 500 μM Aldehyde, and 0.4 Mol % Antibody (2 μM). R = 4-Acetamidophenyl, R′ = 4-Nitrophenyl

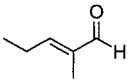
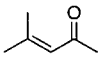
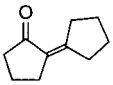
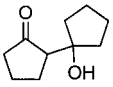
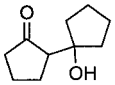
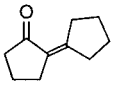
acceptor	donor	product(s)		specific rate
 **3**	acetone		(**18**)	18.1
	cyclopentanone		(**19**)	0.4
	hydroxyacetone		(**20**)	71.6
 **5**	acetone		(**21**)	21.5
	cyclopentanone		(**22**)	15.9
	hydroxyacetone		(**23**)	42.5
 **6**	acetone		(**24**)	4.3
	cyclopentanone		(**25**)	142.1
	hydroxyacetone		(**26**)	103.9
 **7**	acetone		(**27**)	92.3
	cyclopentanone		(**28**)	82.1
	hydroxyacetone		(**29**)	219.9
 **8**	acetone		(**30**)	35.9
	cyclopentanone		(**31**)	11.1
	hydroxyacetone		(**32**)	96.2

suppress the self-aldol addition, a lower concentration of the donor and a higher concentration of the acceptor aldehyde was used. An interesting situation was observed with acetaldehyde. It was found to act solely as an acceptor to give 2-methyl-2-butenal (**50**) with propionaldehyde as donor. 2-Hexenal, the cross-aldol condensation product of the reversed reactivity (acetaldehyde as donor and propionaldehyde as acceptor), was not detected. As shown in Table 6, the antibodies catalyze cross-aldol reactions where simple aliphatic aldehydes act as acceptor substrates. The cross-aldol reaction between cyclopentanone and pentanal yielding **53** proved to be very efficient and kinetic studies revealed a k_{cat} of 1.1 min^{-1} and a K_M for pentanal of 3.9 mM. Pentanal was a very efficient acceptor substrate when paired with hydroxyacetone as the donor. Retroaldol reactions involving the pentanal derived products **55** and **56** were also catalyzed.

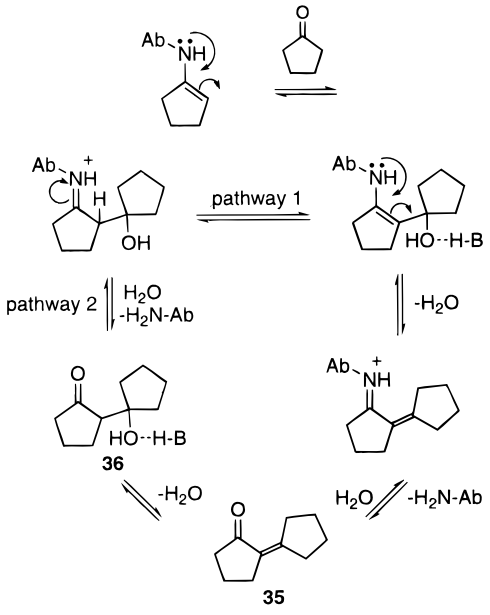
Stereoselectivity and Absolute Configurations of the Aldol Products. Both aldolase antibodies 38C2 and 33F12 catalyze highly stereoselective aldol reactions. As a general rule, acetone adds to aldehydes with *si*-facial selectivity whereas with hydroxyacetone a reversal of enantioface selectivity results in addition to the *re*-face. The products were formed in ee's up to >99% (Table 7). The ee's were determined by HPLC and using chiral stationary phases. To assign absolute configurations, the products were synthesized by either of two ways. In case of the acetone products, we used the method developed by Paterson et al.,[9] utilizing the diisopinocamphenyl enol borinate, prepared in situ from (−)-Ipc$_2$BOTf and acetone. The

(9) Paterson, I.; Goodman, J. M.; Lister, M. A.; Schumann, C.; McClure, C. K.; Norcross, R. D. *Tetrahedron* **1990**, *46*, 4663.

Table 4. Specific Rates [μmol product $\times$ d^{-1} $\times$ μmol^{-1} ab] for Antibody-Catalyzed Self-Aldol Reactions of Propionaldehyde, Acetone, and Cyclopentanone and for the Elimination of Water from **36** to **35** under the Following Defined Conditions: 0.1 M of Ketone or Aldehyde and 0.005 Mol % Antibody (5 μM). These Reactions Take Place Predominantly in the Absence of an Aldehyde Acceptor for Cross-Aldol Reactions

substrate	product/intermediate	specific rate
	 **33**	97.4
	 **34**	< 0.1
	 **35**	0.1
	 **36**	10.3
 **36**	 **35**	4.3

Scheme 2. Proposed Mechanism (pathway 1) for the Antibody-Catalyzed Self-Aldol Condensation of Cyclopentanone. Both Steps, the Addition and the Elimination, Are Catalyzed by the Antibodies. The Less Favored Mechanism (pathway 2) in Which Water Elimination Occurs via Free **36** Is Also Shown



hydroxyacetone products were prepared from the corresponding α,β-unsaturated ketones via Sharpless asymmetric dihydroxylation.[10]

The highest enantioselectivities were observed for aldol reactions where conjugated aldehydes, allylic or benzylic, served as acceptors with acetone as donor. In these cases the observed enantioselectivities were always over 98% ee with either antibody catalyst. Lower enantioselectivities were obtained with aldol acceptors containing an sp^3 center in the α-position, though

(10) (a) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483. Walsh, P. J., Sharpless, K. B. *Synlett* **1993**, 605.

Scheme 3. Diketones **37**, **39**, and **41** Tested as Substrates in the Antibody-Catalyzed Intramolecular Aldol Condensation. Only the *6(enolendo-trig)-exo-trig* Process, Leading to **40** from **39**, Is Catalyzed

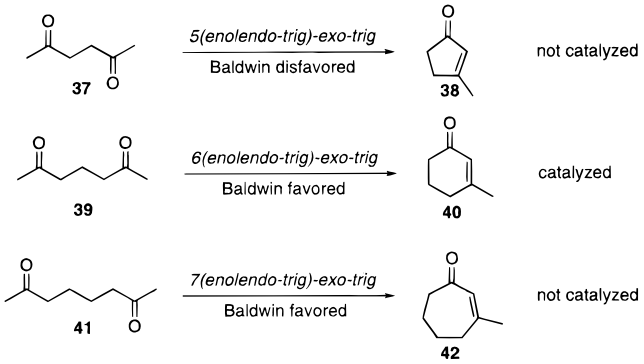
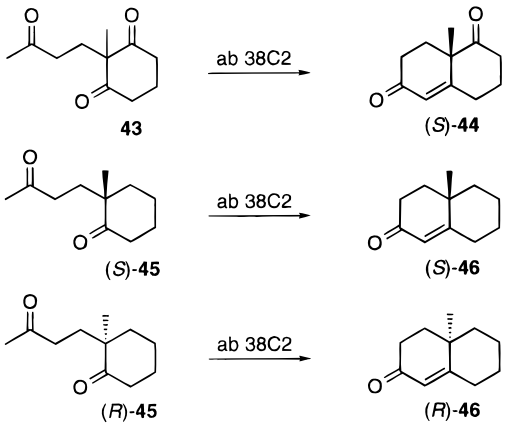


Table 5. Kinetic Parameters (Michaelis−Menten Kinetics) for Intramolecular Aldol Condensations of Substrates **39**, **43**, and **45**

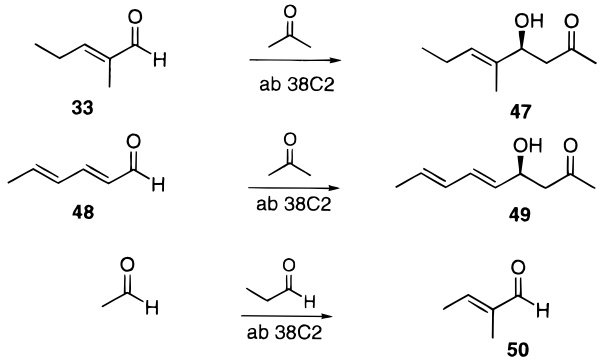
substrate	K_M [mM]	k_{cat} [min^{-1}]	k_{uncat} [min^{-1}]	k_{cat}/k_{uncat}
39	2.05	0.082	6.7×10^{-9}	1.2×10^7
43	2.34	0.086	2.4×10^{-8}	3.6×10^6
(*S*)-**45**	12.4	0.186	nd[a]	nd[a]
(*R*)-**45**	2.45	0.126	nd[a]	nd[a]

[a] Not determined.

Scheme 4. Antibody-Catalyzed Synthesis of Steroid Partial Structures (*S*)-**44** (Wieland−Miescher ketone), (*S*)-**46**, and (*R*)-**46**

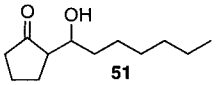
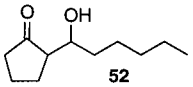
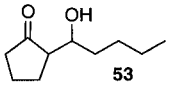
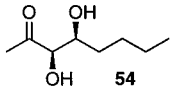
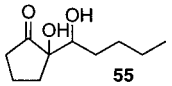
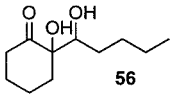


Scheme 5. Aldehydes **33**, **48**, and Acetaldehyde as Acceptors in the Antibody-Catalyzed Cross-Aldol Reactions



enantioselectivity may be increased by addition of steric bulk at this carbon center as suggested with compound **21**. With acceptors of this class, the two catalysts exhibited differential degrees of stereoselectivity. The lowest degree of enantioselectivity is obtained with 4-(4′-acetamidophenyl)butyraldehyde

Table 6. Specific Rates [μmol product $\times$ d^{-1} $\times$ μmol^{-1} ab] for Antibody-Catalyzed Cross-Aldol Reactions of Cyclopentanone and Hydroxyketones with Aliphatic Aldehydes under the Following Defined Conditions: 1 M Donor, 500 μM Aldehyde, and 0.4 Mol % Antibody (2 μM)

acceptor	donor	product	specific rate
n-C_6H_{13}CHO	cyclopentanone	 **51**	17
n-C_5H_{11}CHO	cyclopentanone	 **52**	10
n-C_4H_9CHO	cyclopentanone	 **53**	115
n-C_4H_9CHO	hydroxyacetone	 **54**	nd[a]
n-C_4H_9CHO	2-hydroxy-cyclopentanone	 **55**	nd[a]
n-C_4H_9CHO	2-hydroxy-cyclohexanone	 **56**	nd[a]

[a] Not determined.

as an acceptor and acetone as a donor to yield compound **24**. The ee in this case is 20% and 3%, with catalysts 38C2 and 33F12, respectively. Donor, acceptor, and catalyst all exert an effect on the enantioselectivity of the reaction since use of hydroxyacetone as an aldol donor instead of acetone in the reaction with 4-(4′-acetamidophenyl)butyraldehyde provides product **58** in 77% ee. The regioselectivity of this reaction with respect to bond formation at either α position on hydroxyacetone is perfect. Only product formed by reaction at the α-hydroxy bearing side of hydroxyacetone was detected. Study of the reaction of fluoroacetone with aldehyde **4** catalyzed by 38C2 also demonstrated regioselectivity of bond formation toward the α-substituted carbon of fluoroacetone; however, 7% of the other regioisomer **60** was also isolated (Scheme 6). The *syn*-**16**, constituted 72% of the isolated product and was formed in 95% ee, while the *anti*-**16**, 21% of the isolated product, was formed with 34% ee. The absolute stereochemistry of these products remains to be assigned. The regioselectivity of the aldol addition of fluoroacetone catalyzed by these antibodies is opposite that observed with the natural aldolase, deoxyribose-5-phosphate aldolase, where only addition to the unsubstituted side of fluoroacetone was observed.[11] There are no known catalysts or general methodologies for the asymmetric synthesis of α-fluoro-*β*-hydroxy ketones such as *syn*-**16**.[12] Stereoselectivity involving reactions of α-aliphatic substituted ketones, for example, cyclopentanone, were not studied in detail in this case due to the stereochemical lability at the α-position of products formed with these donors in buffered aqueous solvent.[13] The anti/syn selectivity of reactions with cyclopentanone as the donor in reaction with aliphatic ketones are given in Supporting Information as compared to the product distribution obtained with kinetically controlled synthesis (LDA, −78 °C). With *n*-pentanal as the acceptor, **53** is produced via antibody catalysis with an anti/syn ratio nearly identical to that obtained under kinetically controlled synthesis. A reversal of selectivity is noted with heptanal as the acceptor where the syn-product predominates.

Retroaldolization, Catalytic Efficiency, and Proposed Mechanism. Previous biochemical studies of both catalysts and structural studies of 33F12 are all consistent with an enamine mechanism shared with the natural class I aldolase enzymes.[4] Central to this mechanism is a chemically unique lysine residue bearing an ϵ-amino group with a highly perturbed pK_a allowing for efficient amine-based catalysis under conditions where a more typical amine would be protonated and ineffective in this chemistry. The catalytic efficiency of antibody 38C2 as an aldolase is most readily compared with simple amine catalysis by study of the retroaldol reaction since the second-order rate constant of the amine-catalyzed reaction can be directly related to k_{cat}/K_m of the antibody-catalyzed reaction. Kinetic studies of amine-catalyzed aldol addition reactions have been reported and are approximately 10^2-fold slower than the amine-catalyzed retroaldol reaction.[14] We studied the retroaldol reaction of substrate **59** under antibody and amine catalysis where amine catalysis was studied in both, buffered aqueous solvent and organic solvent (Scheme 7). Antibody 38C2 catalyzed the retroaldolization of **59** following Michaelis−Menten kinetics-($k_{cat} = 1.4$ min^{-1}, $K_m = 270$ μM). The background rate of this retroaldol reaction (100 mM MOPS buffer, pH 7) was determined to be 8.3×10^{-8} min^{-1}. The relative rate enhancement over background provided by the antibody for this reaction (k_{cat}/k_{uncat}) is 1.7×10^7, and the specificity constant (k_{cat}/K_m) is 5.2×10^3 min^{-1} M^{-1}. To date, the most efficient substrate for the 38C2-catalyzed retroaldol reaction is 6-(4′-(dimethylamino)-

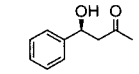
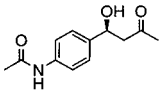
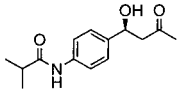
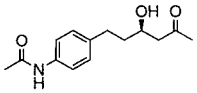
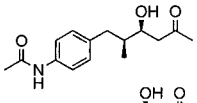
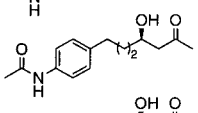
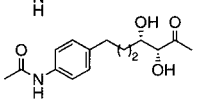
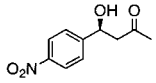
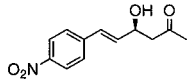
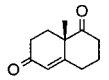
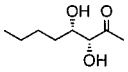
(11) Barbas III, C. F., Wang, Y.-F., Wong, C.-H. *J. Am. Chem. Soc.* **1990**, *112*, 2013.

(12) Welch, J. T.; Seper, K.; Eswarakrishnan, S.; Samartino, J. *J. Org. Chem.* **1984**, *49*, 4720. House, H. O.; Fischer, W. F.; Gall, M.; McLaughlin, T. E.; Peet, N. P. *J. Org. Chem.* **1971**, *36*, 3429−3437. Kowalski, C.; Creary, X.; Rollin, A. J.; Burke, M. C. *J. Org. Chem.* **1978**, *43*, 2601.

(13) Iriye, R.; Takai, K.; Noguchi, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 199. Konieczny, M. T.; Toma, P. H.; Cushman, M. *J. Org. Chem.* **1993**, *58*, 4619.

(14) Reymond, J.-L.; Chen, Y. *J. Org. Chem.* **1995**, *60*, 6979.

Table 7. Stereochemical Purity of Some Products As Determined by Chiral Phase HPLC and GC

product		ee	
		38C2	33F12
	(**57**)	> 99 %	> 99 %
	(**18**)	98 %	99 %
	(**59**)	> 99 %	> 99 %
	(**9**)	58 %	69 %
	(*S*-**21**)	*de* > 95 %*	*de* > 95 %*
	(**24**)	20 %	3 %
	(**58**)	77 % (*de* > 99 %)	70 % (*de* > 99 %)
	(**27**)	98 %	99 %
	(**30**)	99 %	98 %
	*S*-(**44**)	> 95 %	> 95 %
	(**54**)	> 98 %	89 %

*Reference 4a.

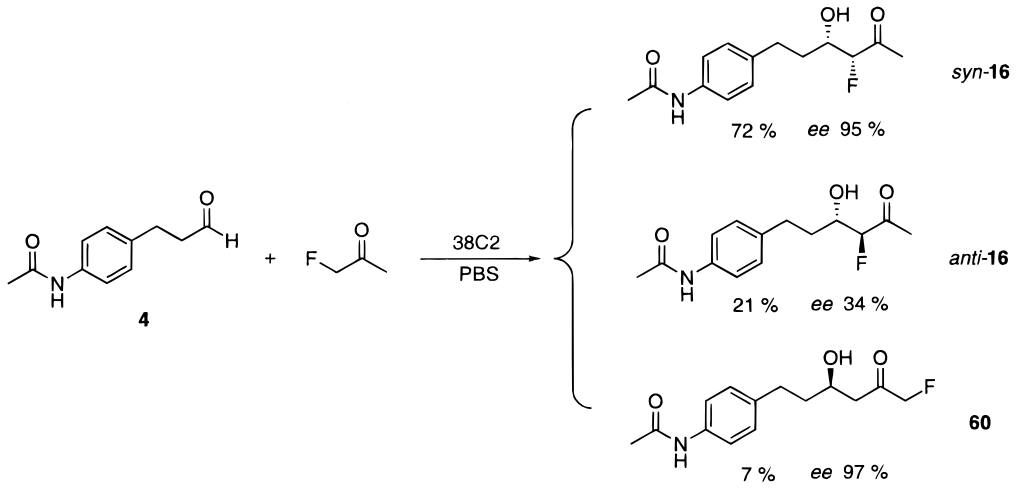
phenyl)-4-hydroxy-5-hexen-2-one. The specificity constant for this substrate is 2.0×10^5.[4c] The specificity constant of antibody 38C2 for this reaction exceeds that previously reported for an amine cofactor-dependent antibody aldolase by a factor greater than 10^6.[15]

Earlier studies have suggested that the pK_a of the ϵ-amino group of the active site lysine, Lys H93, that is central to the chemistry of these catalysts, is highly perturbed by a hydrophobic microenvironment that disfavors protonation and charge development in the unliganded antibodies. The pK_a's of the active-site lysines of 38C2 and 33F12 have been estimated to be 6.0 and 5.5, respectively.[4c] The pK_a of the ϵ-amino group of lysine free in solution is 10.5.[16] These studies also suggested an active-site hydrophobicity that approximates that of *n*-octanol.[4c] Therefore the second-order rate constants for *n*-butylamine- and aminoacetonitrile-catalyzed retroaldolization of **59** were determined in aqueous buffer and *n*-octanol. *N*-Butylamine was studied because of its structural similarity to the side-chain of lysine that is key in the antibody-catalyzed reaction, and aminoacetonitrile was studied since its pK_a approximates that of the active-site lysine of the antibody. The pK_a's of *n*-butylamine and aminoacetonitrile in water are 10.61 and 5.34.[17] The relative efficiency of 38C2 over amine catalysis (k_{cat}/K_m)/k_{NH_2} and the effective molarity (k_{cat}/k_{NH_2}) of the active site lysine of 38C2 are given in Table 8. Antibody 38C2 provides a 10^6−10^8-fold enhancement of the efficiency of the retroaldol reaction of **59** as compared to the nonenzymic amine-catalyzed reactions in either aqueous or organic media. The relative efficiency of 38C2 over simple amine-catalyzed retroaldolization of **59** compares favorably with the efficiency of the enzyme acetoacetate decarboxylase that has been compared with aminoacetonitrile-catalyzed decarboxylation of the same substrate, acetoacetate, where (k_{cat}/K_m)/k_{NH_2} is 9.5×10^6.[18] Acetoacetate decarboxylase is the most studied of enzymes whose mechanism centers around an activated ϵ-amino group of lysine. The effectiveness of the active site amine of 38C2 is also indicated by effective molarities between 560 and 35000 M depending on the amine and solvent system studied. As shown in Table 8, *n*-butylamine catalysis in *n*-octanol is increased 63-fold compared to catalysis in aqueous solution since the amine is in its reactive unprotonated state in *n*-octanol. Aminoacetonitrile exhibits similar efficiency in both solvents due to its low pK_a. The activation energy for this reaction should be lower in nonpolar solvents such as the active site of the antibody and *n*-octanol since the reaction involves charge dispersal in the transition state. A polar medium would be expected to stabilize the cationic iminium intermediates to a greater extent than the activated complex.[19]

The crystal structure of unliganded 33F12 shows LysH93 within hydrogen bonding distance to a water molecule that is also within hydrogen bonding distance to the hydroxyl group of TyrL41. With the caveat that antibodies may undergo large conformational changes on binding, a mechanism analogous to that proposed for class I aldolases is shown in Figure 2 and appears most likely. In the proposed mechanism, TyrL41 may act as the base. Acceptor activation appears to be essential since we have failed to trap the enamine with addition of alkylating substrates such as 4-nitrophenethyl bromide or α,β-unsaturated substrates such as methyl vinyl ketones according to Stork enamine[20] or Michael reactions.[21] Acceptor activation and the microenvironment in which it occurs, however, must be balanced so as not to facilitate formation of the unreactive *gem*-diol form of the aldehyde. Enamine formation and or the C−C bond-forming/breaking step are rate-limiting with these catalysts. Study of the 38C2-catalyzed retroaldolization of **59** in ^{18}O-labeled water by electrospray mass spectrometry showed rapid antibody-catalyzed incorporation of ^{18}O into substrate **59** (Figure 3). Within 6 min, 1 mol % 38C2 catalyzed ^{18}O incorporation into more than 50% of the substrate **59** molecules where no incorporation in the control reaction not containing antibody

(15) Reymond, J.-L. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2285.
(16) Dean, J. A. In *Lange's Handbook of Chemistry*; McGraw-Hill: San Francisco, CA, 1992; pp 8.19−8.71.
(17) Hupe, D. J.; Kendall, M. C. R.; Spencer, T. A. *J. Am. Chem. Soc.* **1972**, *94*, 1254.
(18) Westheimer, F. H. *Proceeding of the Robert A. Welch Found. Conf. Chem. Res.* **1971**, *15*, 7. Westheimer, F. H. *Tetrahedron* **1995**, 51, 3. Highbarger, L. A.; Gerlt, J. A.; Kenyon, G. L. *Biochemistry* **1996**, *35*, 41.
(19) Reichardt, C. In *Solvents and Solvents Effects in Organic Chemistry*, 2nd ed.; Ebel, H. F., Wentrup-Byne, E., Eds.; New York, 1990; pp 1−511. Heathcock, C. H. Stereoselective Aldol Condensations. In *Comprehensive Carbanion Chemistry*; Buncel, E., Durst, T., Eds.; Elsevier: Amsterdam, 1984; part B, p 177ff (particularly p 198).
(20) Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrell, R. *J. Am. Chem. Soc.* **1963**, *85*, 207. For a general review including experimental procedures, see: Whitesell, J. K., Whitesell, M. A. *Synthesis* **1983**, 517−536.
(21) Lucero, M. J.; Houk, K. N. *J. Am. Chem. Soc.* **1997**, *119*, 826. For leading references, see: Pfau, M.; Revial, G.; Guingant, A.; d'Angelo, J. J. *J. Am. Chem. Soc.* **1985**, *107*, 273. d'Angelo, J.; Desmaële, D.; Dumas, F.; Guingant, A. *Tetrahedron: Asymmetry* **1992**, 3, 459. Jabin, I.; Revial, G.; Tomas, A.; Lemoine, P.; Pfau, M. *Tetrahedron: Asymmetry* **1995**, 6, 1795. Cavé, C.; Desmaële, D.; d'Angelo, J. *J. Org. Chem.* **1996**, *61*, 4361.

Scheme 6. Preparation of Fluorinated Aldols (*syn*-**16**, *anti*-**16**, and **60**) by Antibody-Catalyzed Aldol Reaction



Scheme 7. Antibody- or Amine-Catalyzed Retroaldol Reaction of Substrate **59**

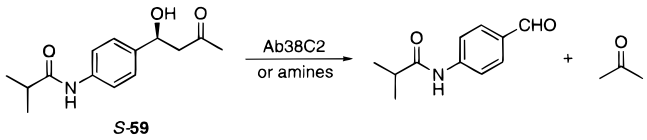


Table 8. Comparison of Antibody 38C2-Catalyzed Retroaldolization of **59** with Amine-Catalyzed Retroaldolization in Aqueous and Organic Solvents

amine/solvent	$(k_{cat}/K_m)^a/k_{amine}$	k_{cat}/k_{amine} (M)
n-butylamine/MOPS[b]	1.3×10^8	3.5×10^4
n-butylamine/*n*-octanol	2.1×10^6	5.6×10^2
aminoacetonitrile/MOPS[b]	2.9×10^7	7.8×10^3
aminoacetonitrile/*n*-octanol	1.6×10^7	4.2×10^3

[a] k_{cat} and K_m of antibody 38C2-catalyzed retroaldolization (Scheme 7). [b] 100 mM MOPS buffered water, pH 7.0, 25 °C.

was seen at this time point. This result contrasts what we observed in similar studies of 38C2-catalyzed decarboxylation of a β-keto acid where imine formation is rate-limiting and catalysis of ^{18}O exchange back into the substrate was not observed.[5] Reactions involving hydroxyacetone as donor are faster than those involving acetone. This observation is also in support of enamine formation being at least partially rate-determining since ab initio molecular orbital calculations predict a lower energy of activation for the imine to enamine interconversion for hydroxyacetone relative to acetone by approximately 2 kcal/mol due to the electron-withdrawing character of the hydroxy group on hydroxyacetone that facilitates proton abstraction at the α-carbon.[22] This together with an increased relative stability of the enamine formed at the hydroxy-bearing side of hydroxyacetone explains the perfect control of the regiochemistry of hydroxyacetone aldols where bond formation has only been detected at the α-position bearing the hydroxyl group. By similar arguments, the observed regioselectivity of fluoroacetone addition is also in accord with these reported ab initio molecular orbital calculations.[22] The reversal in the enantiofacial selectivity of the addition of hydroxyacetone remains to be completely explained. The α-syn stereochemistry of the hydroxyacetone-derived products **54** and **58** can be rationalized by the preferential formation of the *Z*-enamine of hydroxyacetone, stabilized over the *E*-configuration via intramolecular hydrogen bonding, and subsequent attack on the

(22) Lin, J.-F.; Wu; C.-C.; Lien, M.-H. *J. Phys. Chem.* **1995**, *99*, 16903.

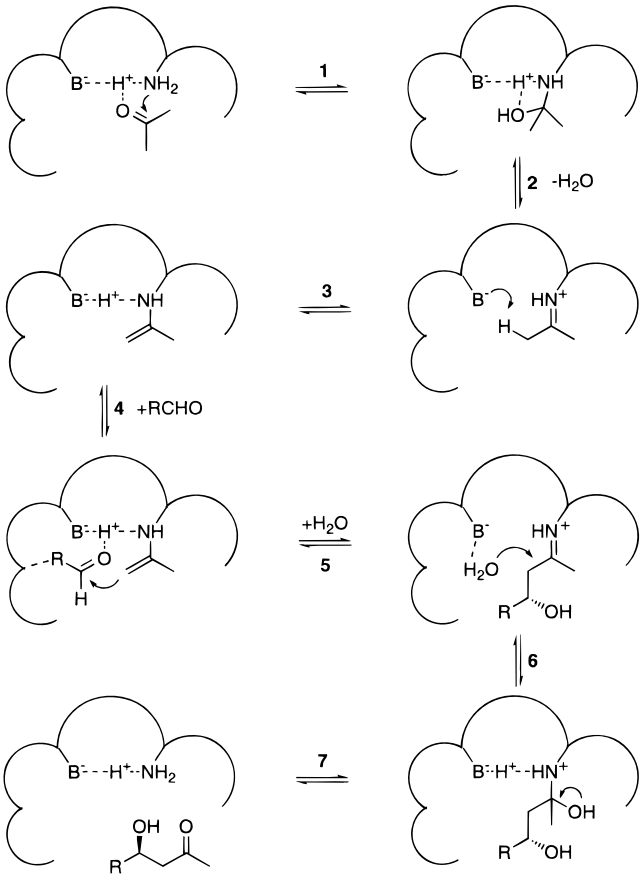


Figure 2. Detailed representation of the suggested mechanism of ab 38C2 and 33F12 catalysis of the aldol reaction. The rate-determining step is presumably the C−C bond formation in step 5.

re-face of the acceptor as shown in Figure 4. Branching at the α-position of the enamine may result in a reorganization of an activating water molecule or another amino acid side-chain that serves this function, altering the enantiofacial selectivity. The observation of both, syn and anti products, in additions of cyclopentanone to aldehydes, products **51**−**53**, supports the availability of both faces of the acceptor aldehyde toward attack with this enamine which is limited to formation of an *E*-enamine. Diminished enantioselectivity with substrates such as **6** over the high enantioselectivities observed with benzylic or allylic acceptors **3**, **7**, and **8** is consistent with more efficient enantioface selectivity with acceptor molecules with fewer degrees of

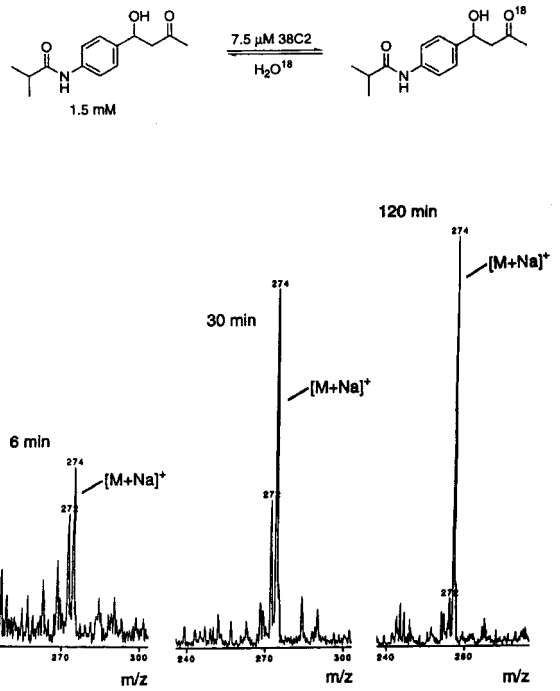


Figure 3. Mass spectra from electrospray MS showing the antibody-catalyzed O-exchange of the aldol product **59** in buffered 98% ^{18}O-labeled water. The *m*/*z* of 272 corresponds to the starting material whereas the antibody-catalyzed exchange (presumably via a covalently bound intermediate) incorporated ^{18}O to produce *m*/*z* of 274. Negligible ^{18}O incorporation into **59** was observed in the absence of ab 38C2 under otherwise identical conditions.

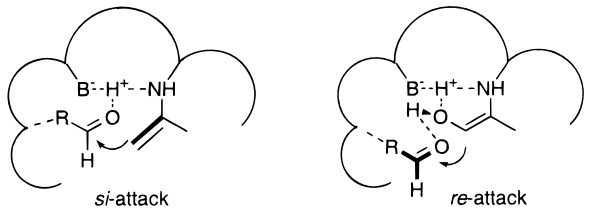
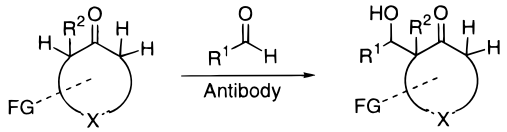


Figure 4. Potential mechanistic origin of enantioselectivities where acetone and hydroxyacetone serve as aldol donor substrates.

freedom. Further insight into the chemical mechanism of these catalysts will come with the solution of the X-ray crystal structure of antibody 33F12 bound to hapten **1**. This study is now underway.

Conclusions. Antibodies 38C2 and 33F12 are capable of efficiently catalyzing a wide variety of ketone−ketone, ketone−aldehyde, aldehyde−ketone, and aldehyde−aldehyde intermolecular aldol reactions, and in some cases to catalyze their subsequent dehydration to yield aldol condensation products. A number of intramolecular aldol reactions have also been defined. Catalysis of all intramolecular aldol reactions examined yields the corresponding condensation products. The consensus donor and acceptor substrates are given in Figure 5. The primary limitation in donor specificity appears to be an inability of the catalysts to accept ketones that are branched at the non-bond-forming α-position, whereas acceptor substrates are only limited to the extent that they are relatively hydrophobic aldehydes or ketones since no polyhydroxylated aldehydes have yet been defined as substrates. The scope of these antibody catalysts exceeds that observed with any known natural enzyme aldolase or transition metal based aldol catalysts.[2,3] When acetone is the aldol donor substrate in a ketone−aldehyde crossed aldol reaction, a new stereogenic center is formed by attack of the *si*-face of the aldehyde with ee's in most cases



R^1 = (FG)-alkyl, (FG)-alkenyl, (FG)-aryl
R^2 = H, OH, F
X = NCH_3, O, S, CH_2, C_6H_4
FG = OH, OCH_3

Figure 5. Consensus ketone−aldehyde cross-aldol substrates for antibodies 38C2 and 33F12.

exceeding 95%. With hydroxyacetone as the donor substrate, attack occurs at the *re*-face of the aldehyde generating an α,*β*-dihydroxy ketone with the two stereogenic centers having a α-syn configuration, (3*R*,4*S*). These reactions proceed with 70 to >99% ee. The major product of a cross-aldol reaction with fluoroacetone as the donor substrate is a syn α-fluoro-*β*-hydroxy ketone formed in 95% ee.

These studies highlight the ability of reactive immunization to produce catalysts that are efficient yet broad in scope. Since such antibodies are tailor-made and optimized by the immune system to covalently bind the hapten, the binding pocket is not necessarily refined with respect to noncovalent interactions with the immunogen.[4c] Consequently, the biocatalyst can accept a variety of substrates which differ enormously with respect to their physicochemical properties. Antibodies 38C2 and 33F12 are useful additions to the repertoire of asymmetric catalysts due to their tremendous scope, efficiency, and stereoselectivities. This has been demonstrated herein with syntheses up to the 1 g scale and in the highly enantioselective total syntheses of 10 different brevicomins.[23] Unlike current transition metal catalysts, the antibodies reported here catalyze both, the aldol and retroaldol reaction, allowing for the preparation of both enantiomers of *β*-hydroxy ketones with high optical purity either by enantioselective synthesis or kinetic resolution.[24] The issue of scale in antibody-catalyzed reactions can be addressed with currently available technologies, particularly the heterologous expression of antibodies in plants and algaes, where low-cost production of these catalysts on a multiton scale could be achieved to allow for the "green synthesis" of aldols on a virtually unlimited scale.[25] Antibody 38C2 is now commercially available from the Aldrich Chemical Co.

Experimental Section

General. All reactions requiring anhydrous conditions were performed in oven-dried glassware under an Ar or N_2 atmosphere. Chemicals and solvents were either *puriss p.A.* or purified by standard techniques. THF was distilled from sodium−benzophenone. Thin-layer chromatography (TLC): silica gel plates Merck 60 F_{254}, compounds were visualized by irradiation with UV light and/or by treatment with a solution of 25 g of phosphomolybdic acid, 10 g of $Ce(SO_4)_2 \cdot H_2O$, 60 mL of concd H_2SO_4, and 940 mL of H_2O followed by heating and/or by staining with a solution of 12 g of 2,4-dinitrophenylhydrazine in 60 mL of concd H_2SO_4, 80 mL of H_2O, and 200 mL of 95% EtOH followed by heating and/or by immersing in an iodine bath (30 g of I_2, 2 g of KI, in 400 mL of EtOH/H_2O 1:1) and warming. Flash chromatography (FC): silica gel Merck 60 (particle size 0.040−0.063

(23) List, B.; Shabat, D.; Barbas III, C. F.; Lerner, R. A. *Chem. Eur. J.* In press.
(24) Zhong, G. et al., submitted.
(25) Hiatt, A. *Nature* **1990**, *334*, 469. Hiatt, A.; Ma, J. K. *Int. Rev. Immunol.* **1993**, *10*, 139. Ma, J. K.; Hiatt, A.; Hein, M.; Vine, N. D.; Wang, F.; Stabila, P.; van Dolleweerd, C.; Mostov, K.; Lehner, T. *Science* **1995**, *268*, 716. Franken, E.; Teuschel, U.; Hain, R. *Curr. Opin. Biotechnol.* **1997**, *8*, 411.

mm), eluent given in parentheses. ^{1}H NMR: Bruker AMX 300, Bruker AMX 250. The chemical shifts are given in δ relative to TMS (δ = 0 ppm), the coupling constants J are given in Hz. The spectra were recorded in $CDCl_3$ as solvent at room temperature unless stated otherwise. HR-MS: liquid secondary ionization (LSI-MS): VG ZAB-ZSE with 3-nitrobenzyl alcohol matrix.

Antibody Stability. The antibodies 38C2 and 33F12 are stable at room temperature for weeks dissolved in different buffer solutions (pH 5.5 to 8.5) and even pure water. They can be lyophilized and passed over a Sephadex column with less than 5% activity loss. No detectable activity loss was found if the antibodies were stored in stock solutions of 10 to 20 mg/mL in phosphate-buffered saline (PBS) (10 mM phosphate, 150 mM NaCl, pH 7.4) at −78 °C.

Preparation of 4-(4′-Acetamidophenyl)butyraldehyde (6). Aldehyde **6** was prepared in four steps starting from commercially available 4-(4′-aminophenyl)butyric acid as follows.

(i) 4-(4′-Acetamidophenyl)butyric Acid. 4-(4′-Aminophenyl)-butyric acid (4.0 g, 22 mmol) was added to 150 mL of mixed solvent of acetonitrile and water (9/1). Acetic anhydride (7.4 g, 55 mmol, 2.5 equiv) was added at 0 °C. Then the reaction mixture was stirred at room temperature for 4 h. The filtration of the reaction mixture was followed by drying at 120 °C overnight to give 4.53 g of 4-(4′-acetamidophenyl)butyric acid (93%). HR-MS: 222.1140; $C_{12}H_{16}O_3N^+$ (calcd 222.1130); $C_{12}H_{15}O_3N$ (221.26).

(ii) 4-(4′-acetamidophenyl)butyric Acid Methyl Ester. 4-(4′-Acetamidophenyl)butyric acid (4.50 g, 20 mmol) and potassium carbonate (2.90 g, 21 mmol) were added to 20 mL of dry DMF. The reaction mixture was stirred at room temperature for 15 min. Then methyl iodide (14.2 g, 0.10 mol) was added under nitrogen. The reaction mixture was stirred at room-temperature overnight. After evaporation of the solvent and methyl iodide, a solid mixture was obtained, from which the methyl ester was isolated by extraction with ethyl acetate (3 × 70 mL) to 4.7 g (>99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (d, J 8.4, 2 H), 7.34 (s, br, 1 H), 7.12 (d, J 8.4, 2 H), 3.67 (s, 3 H), 2.61 (t, J 7.3, 2 H), 2.32 (t, J 7.3, 2 H), 2.17 (s, 3 H), 1.93 (hept, J 7.3, 2 H); HR-MS: 236.1296; $C_{13}H_{18}O_2N^+$ (calcd 236.1287); $C_{13}H_{17}O_3N$ (235.28).

(iii) 4-(4′-Acetamidophenyl)butanol. 4-(4′-Acetamidophenyl)butyric acid methyl ester (4.70 g, 20 mmol) was dissolved in 50 mL of dry THF, and DIBALH in methylene chloride (1.0 M, 40 mL) was dropwise added at −30 °C. The mixture was kept stirring at this temperature for 3 h. Saturated ammonium chloride (25 mL) was added slowly. Extraction with 3 × 80 mL of ethyl acetate followed by evaporation of the solvent gave a residue which was purified by FC (hexane/ethyl acetate 4:1) to give 2.73 g (66%) of 4-(4′-acetamidophenyl)butanol. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42 (d, J 8.4, 2 H), 7.23 (s, br, 1 H), 7.13 (d, J 8.4, 2 H), 3.71 (t, J 7.2, 2 H), 2.56 (t, J 7.2, 2 H), 2.17 (s, 3 H), 1.65 (m, 4 H); HR-MS: 208.1342; $C_{12}H_{18}O_2N^+$ (calcd 208.1338); $C_{12}H_{17}O_2N$ (207.27).

(iv) 4-(4′-Acetamidophenyl)butyraldehyde (6). A mixture of methylene chloride (25 mL) and oxalyl chloride (1.0 mL, 11 mmol) were placed in a flask. Dimethyl sulfoxide (1.7 mL, 22 mmol) diluted with methylene chloride (5 mL) was added to the stirred solution at −78 °C. The reaction mixture was stirred for 2 min, and 4-(4′-acetamidophenyl)butanol (2.2 g, 10 mmol) in 10 mL of methylene chloride was added within 5 min. The stirring was continued for additional 15 min. Triethylamine (7.0 mL, 50 mmol) was added and the reaction mixture was stirred for 5 min and then allowed to warm to room temperature. Water (50 mL) was added, and the aqueous layer was reextracted with methylene chloride (50 mL). The organic layers were combined, washed with saturated sodium chloride solution (100 mL), and dried with magnesium sulfate. After concentration, 2.07 g (94%) of pure 4-(4′-acetamidophenyl)butyraldehyde (**6**) was obtained by FC (hexane/ethyl acetate 3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.76 (s, br, 1 H), 7.42 (d, J 8.4, 2 H), 7.23 (s, br, 1 H), 7.13 (d, J 8.4, 2 H), 2.63 (t, J 7.2, 2 H), 2.45 (t, J 7.2, 2 H), 2.17 (s, 3 H), 1.94 (hept, J 7.2, 2 H); HR-MS: 206.1185; $C_{12}H_{16}O_2N^+$ (calcd 206.1181); $C_{12}H_{15}O_2N$ (205.26).

General Procedure for the Preparation of Aldol Products. The corresponding ketone (1.0 mmol) was added to a freshly prepared solution of LDA (1.05 mmol) in 2 mL of THF at −78 °C. After stirring at this temperature for 30 min, the aldehyde (1.0 mmol), dissolved in 2 mL of THF, was added over a period of 1 min. After stirring for 5−30 min at −78 °C, saturated NH_4Cl solution (1 mL) was added, and the reaction mixture was allowed to warm to room temperature. The product was extracted with ethyl acetate (3 × 10 mL), dried ($MgSO_4$), and evaporated. The pure aldol products were obtained by FC. Spectroscopic data for the acetone addition products with aldehydes **3**−**8** and **48** are given below as examples.

6-(4′-Acetamidophenyl)-4-hydroxy-2-hexanone (9). FC (ethyl acetate/hexane 75:25) gave 0.21 g (85%) of pure aldol **9**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12 (s, br, 1 H), 7.37 (d, J 8.4, 2 H), 7.06 (d, J 8.4, 2 H), 4.01 (m, 1 H), 3.42 (m, 1 H), 2.71 (m, 1 H), 2.62 (m, 3 H), 2.13 (s, 3 H), 2.10 (s, 3 H), 1.75 (m, 1 H), 1.64 (m, 1 H); HR-MS: 250.1450; $C_{14}H_{20}O_3N^+$ (calcd 250.1443); $C_{14}H_{19}O_3N$ (249.31).

4-(4′-Acetamidophenyl)-4-hydroxy-2-butanone (18). FC (ethyl acetate/hexane 70:30) gave 0.18 g (82%) of pure aldol **18**. ^{1}H NMR (250 MHz, DMSO-d_6): δ 9.88 (s, 1 H), 7.49 (d, J 8.5, 2 H), 7.24 (d, J 8.5, 2 H), 5.28 (d, J 4.4, 1 H), 4.91 (m, 1 H), 2.65 (m, 2 H), 2.09 (s, 3 H), 2.01 (s, 1 H); HR-MS: 244.0955; $C_{12}H_{15}O_3NNa^+$ (calcd 244.0949); $C_{12}H_{15}O_3N$ (221.26).

4-(4′-Isobutyramidophenyl)-4-hydroxy-2-butanone (59). FC (ethyl acetate/hexane 40:60) gave 0.23 g (91%) of pure aldol **59**. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.50 (d, J 8.5, 2 H), 7.30 (d, J 8.5, 2 H), 7.27 (s, br, 1 H), 5.12 (m, 1 H), 3.36 (s, 1 H), 2.84 (m, 2 H), 2.51 (pent, J 6.8, 1H), 2.20 (s, 3 H), 1.25 (d, J 6.8, 6 H); HR-MS: 272.1267; $C_{14}H_{19}O_3NNa^+$ (calcd 272.1263); $C_{14}H_{19}O_3N$ (249.31).

6-(4′-Acetamidophenyl)-4-hydroxy-5-methyl-2-hexanone (21). FC (ethyl acetate/hexane 70:30) gave 0.21 g (79%) of pure aldol **21**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (s, br, 1 H), 7.39 (d, J 8.3, 2 H), 7.07 (d, J 8.3, 2 H), 3.95 (m, 1 H), 3.08 (m, 1 H), 2.82 (m, 1 H), 2.66 (m, 3 H), 2.55 (m, 1 H), 2.37 (m, 1 H), 2.18 (s, 3 H), 2.15 (s, 3 H), 1.76 (m, 1 H), 0.85 (d, J 6.6, 3 H); HR-MS: 264.1608; $C_{15}H_{22}O_3N^+$ (calcd 264.1600); $C_{15}H_{21}O_3N$ (263.34).

7-(4′-Acetamidophenyl)-4-hydroxy-2-heptanone (24). FC (ethyl acetate/hexane 70:30) gave 0.23 g (89%) of pure aldol **24**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (d, J 8.4, 2 H), 7.24 (s, br, 1 H), 7.13 (d, J 8.4, 2 H), 4.05 (m, 1 H), 3.04 (m, 1 H), 2.57 (m, 4 H), 2.17 (s, 3 H), 2.16 (s, 3 H), 1.70 (m, 2 H), 1.45 (m, 2 H); HR-MS: 286.1411; $C_{15}H_{21}O_3NNa^+$ (calcd 286.1419); $C_{15}H_{21}O_3N$ (263.34).

4-(4′-Nitrophenyl)-4-hydroxy-2-butanone (27). FC (ethyl acetate/hexane 50:50) gave 0.18 g (86%) of pure aldol **27**. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.21 (d, J 7.2, 2 H), 7.52 (d, J 7.2, 2 H), 5.25 (m, 1 H), 3.56 (d, J 3.2, 1 H), 2.83 (m, 2 H), 2.21 (s, 1 H); HR-MS: 232.0591; $C_{10}H_{11}O_4NNa^+$ (calcd 232.0586); $C_{10}H_{11}O_4N$ (209.20).

6-(4′-Nitrophenyl)-4-hydroxy-5-hexen-2-one (30). FC (ethyl acetate/hexane 50:50) gave 0.21 g (89%) of pure aldol **30**. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.15 (d, J 7.3, 2 H), 7.46 (d, J 7.3, 2 H), 6.71 (d, J 15.9, 1 H), 6.35 (dd, J 15.9, J 5.3, 1 H) 4.78 (m, 1 H), 3.28 (d, J 3.7, 1 H), 2.73 (m, 2 H), 2.21 (s, 1 H); HR-MS: 258.0751; $C_{12}H_{13}O_4NNa^+$ (calcd 258.0742); $C_{12}H_{13}O_4N$ (235.24).

4-Hydroxynona-5,7-dien-2-one (49). FC (ethyl acetate/hexane 15:85) gave 88 mg (57%) of pure aldol **49**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.21 (m, 1 H), 6.05 (m, 1 H), 5.74 (m, 1 H), 5.54 (m, 1 H), 4.56 (m, 1 H), 2.93 (m, 1 H), 2.62 (d, J 6.2, 2 H), 2.18 (s, 3 H), 1.75 (d, J 6.5, 3 H); HR-MS: 177.0899; $C_9H_{14}O_2Na^+$ (calcd 177.0892); $C_9H_{14}O_2$ (154.21).

Antibody Catalysis. Preparative Antibody-Catalyzed Reactions. Example 1. Preparation of (*S*)-4-Hydroxy-6-(4-nitrophenyl)-5-hexen-2-one (30). To a solution of 4-nitrocinnamaldehyde (110 mg, 0.61 mmol) in 15 mL of DMF and 31 mL of acetone was added PBS buffer (571 mL, degassed and kept under argon) slowly to avoid precipitation. Antibody 38C2 (8.0 mL of a 120 μM solution) was added. The final concentrations of 4-nitrocinnamaldehyde and Ab38C2 were 1.0 mM and 1.9 μM, respectively, in a total volume of 625 mL containing 5% (v/v) of acetone. The reaction mixture was kept in a dark place at room temperature for 7 days under argon. The reaction mixture was saturated with sodium chloride and extracted with 3 × 150 mL of ethyl acetate. The extracts were dried over $MgSO_4$ and evaporated to yield 140 mg of crude product. Purification by FC (1:2, ethyl acetate/hexane) gave 96 mg (67%) of pure aldol product (**30**) with an ee of 91%.

Example 2. Enantioselective Preparation of Fluorinated Aldols. A solution of 3-(4′-acetamidophenyl)propanal **4** (15 mg, 0.078 mmol) in 0.2 mL of DMF, 0.5 mL of fluoroacetone, and 8.0 mL of PBS buffer was added to Ab38C2 (1.5 mL of a 120 μM solution). The final concentrations of 3-(4′-acetamidophenyl)propanal and Ab38C2 were 7.6 mM and 17.5 μM, respectively, in a total volume of 10.2 mL containing 5% (v/v) of fluoroacetone. The reaction mixture was kept at room temperature for 21 days. Three aldol products *syn*-**16** (ee 95%), *anti*-**16** (ee 34%), and **60** (ee 97%) (Scheme 6) were isolated by semipreparative RP-HPLC (column: VYDAC protein and peptide C18, λ = 254 nm, 12% CH_3CN/88% water with 0.1% TFA, 4.0 mL/min, 1.0 mL reaction mixture/injection) to give *syn*-**16** (12 mg, t_R = 20.8 min, yield 61%), *anti*-**16** (3.6 mg, t_R = 18.2 min, yield 21%), and **60** (1.2 mg, t_R = 16.3 min, yield 7%). The overall yield was 82% (15% of aldehyde **4** was recovered).

Syn-isomer of 6-(4′-acetamidophenyl)-3-fluoro-4-hydroxy-2-hexanone (*syn*-16): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (d, *J* 8.4, 2 H), 7.22 (s, br, 1 H), 7.15 (d, *J* 8.4, 2 H), 4.62 (dd, *J* 48.8, 4.9, 2 H), 3.97 (m, 1 H), 2.85 (m, 1 H), 2.67 (m, 1 H), 2.30 (d, *J* 5.1, 3 H), 2.18 (s, 3 H), 1.84 (m, 2 H).

Anti-isomer of 6-(4′-acetamidophenyl)-3-fluoro-4-hydroxy-2-hexanone (*anti*-16): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42 (d, *J* 8.4, 2 H), 7.17 (s, br, 1 H), 7.16 (d, *J* 8.4, 2 H), 4.64 (dd, *J* 48.9, 2.4, 1 H), 4.00 (dm, *J* 24.5, 1 H), 2.77 (m, 2 H), 2.31 (d, *J* 5.0, 3 H), 2.18 (s, 3 H), 1.94 (m, 2 H).

Regioisomer, 6-(4′-acetamidophenyl)-1-fluoro-4-hydroxy-2-hexanone (60): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43 (d, *J* 8.4, 2 H), 7.17 (d, *J* 8.4, 2 H), 7.14 (s, br, 1 H), 4.83 (d, *J* 47.5, 2 H), 4.12 (m, 1 H), 2.73 (m, 4 H), 2.18 (s, 3 H), 1.81 (m, 2 H).

Example 3. Preparative Scale Synthesis of 2-[1′-(4″-Isobutyramidophenyl)-1′-hydroxymethyl]cyclopentanone (61). Cyclopentanone (1 mol, 88 mL) was dissolved in 912 mL of MOPS buffer (100 mM, pH = 7.4). Next, Ab38C2 (1.3 μmol, 0.1 g) was added, and the first addition of aldehyde, 4-isobutyramidobenzaldehyde (1.1 mmol, 213 mg), was made. The reaction was stirred for 24 h followed by a second addition of aldehyde. Two subsequent additions of aldehyde followed at 24 h time periods for a total of 852 mg, 4.4 mmol. The reaction progress was monitored by HPLC (Hitachi HPLC system: pump L-7100, UV detector L-7400, and integrator D-7500) using a Rainin column (Microsorb-MV, C18, 300 Å, 5 mm; 250 × 4.6 mm) and acetonitrile/water mixture (20% CH_3CN/80% water containing 0.1% trifluoroacetic acid) with a flow rate of 1.0 mL/min. The reaction mixture was kept in a dark place at room temperature for 21 days under argon. The reaction mixture was then saturated with NaCl. The mixture was extracted with 3 × 500 mL of ethyl acetate, dried over $MgSO_4$, and evaporated to yield 1.4 g of crude product. Purification by FC (60:40, EtAc/Hex) gave 0.9 g (72%) of pure product **61** with a de of >95%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49 (d, *J* 8.4, 2 H), 7.31 (s, br, 1 H), 7.27 (d, *J* 8.4, 2 H), 5.24 (s, 1 H), 4.62 (m, 1 H), 2.6−1.6 (m, 7 H), 1.26 (d, *J* 6.8, 6 H).

Antibody Assays. All antibody-catalyzed reactions were performed in phosphate buffered saline (10 mM phosphate, 150 mM NaCl, pH 7.4) except reactions with substrate **43** (Scheme 4, H_2O, pH 6.5) and substrates *n*-C_4H_9CHO, *n*-$C_5H_{11}CHO$, *n*-$C_6H_{13}CHO$ (Table 6, (3-morpholinopropanesulfonic acid) sodium salt buffer (MOPS buffer), pH 7.0). All antibody-catalyzed reactions and background reactions with substrates **3**−**8**, **33**, **37**, **39**, **41**, **43**, **45**, and **48** were monitored by high-pressure liquid chromatography (HPLC; Hitachi HPLC system (pump L-7100, UV detector L-7400, and integrator D-7500) using a Rainin column (Microsorb-MV, C18, 300 Å, 5 mm; 250 × 4.6 mm) and acetonitrile/water mixtures (containing 0.1% trifluoroacetic acid) as eluents at a flow rate of 1.5 mL/min or 1.0 mL/min. Formation of products **51**−**53** was followed by gas chromatography (DB-5, J&W Scientific, length: 30 m, i.d.: 0.32 mm, temp: 65 °C (2 min), rate: 10 °C/min, t_R: (*anti*-**51**) = 13.82 min, (*syn*-**51**) = 14.05 min; (*anti*-**52**) = 12.56 min, (*syn*-**52**) = 12.82 min; (*anti*-**53**) = 11.24 min, (*syn*-**53**) = 11.52 min).

Specific Rates of Cross-Aldol Reactions. The specific rates of cross-aldol reactions were determined before 10% completion of the reactions using initial concentrations of the acceptor substrate (500 μM), antibody (2 μM), and donor ketone (1.0 M).

Specific Rates of Self-Aldol and Intramolecular Aldol Reactions. The specific rates of self-aldol and intramolecular aldol reactions were determined before 10% completion of the reactions using initial concentrations of the substrate (0.10 M) and antibody (5 μM).

Michaelis−Menten Kinetics. Product formation or percent conversion of antibody-catalyzed reaction mixtures was monitored by HPLC or GC. The experimental data was plotted using nonlinear regression analysis with GraFit software to give k_{cat} and K_M of the reactions. All data are reported per antibody active site. An IgG antibody possesses 2 active sites per MW of ∼150000 g/mol.

Determination of Enantiomeric Excess of Products 9, 18, 59, 21, 24, 27, 30, and 54. To a 6.25 mM solution of the aldehyde in 160 μL of PBS were added 10 μL of acetone and 30 μL of a 135 μM solution of the antibody in PBS. The final concentrations were 5 mM of aldehyde and 20 μM of antibody in a total volume of 200 mL of PBS containing 5% (v/v) of acetone. After 18 h, 12 mL of CH_2Cl_2 were added, and the organic phase was dried ($MgSO_4$) and evaporated. In case of products **9**, **18**, **59**, **21**, **24**, **27**, and **30** the residue was redissolved in ca. 1 mL of 2-propanol and the ee was determined by normal phase HPLC using an appropriate Daicel column for enantiomer separation. The ee of product **54** was determined using GC on a chiral capillary column (Cyclodex-B, J&W Scientific, length: 30 m, i.d.: 0.25 mm, temp: 150 °C, t_R: (α) = 15.48 min, (β) = 16.01 min).

^{18}O Incorporation. The electron spray ionization (ESI) mass spectrometry used to monitor ^{18}O incorporation into **59** was performed on an API III Perkin-Elmer SCIEX triple quadropole mass spectrometer. In preparation of the sample, lyophilized antibody 38C2 was resuspended in ^{18}O labeled water (^{18}O, 95−98%, Cambridge Isotope Laboratories, Andover, MA) to give a final concentration of 7.5 μM. The reaction was started by addition of aldol product **59** (1.5 mM), and aliquots were taken out for analysis of ^{18}O incorporation over time. Immediately before analysis the samples were diluted 10-fold in methanol.

Acknowledgment. We are grateful to M. G. Finn for helpful discussions, Brian Bothner and Gary Suizdak for assistance with MS, Ron Lewis for studies of Michael reactions, and Gunther Schlingloff as well as other members of the Sharpless laboratory for their help with ee measurements. This study was supported in part by the NIH (CA27489). T.H and B.L. thank the Alexander von Humboldt Foundation, Germany, for a Feodor Lynen fellowship. C.F.B. acknowledges an Investigator Award from the Cancer Research Institute.

Supporting Information Available: Kinetic data, nondonor substrate structures, and diastereoselectivity studies (7 pages). See any current masthead page for ordering and Web access instructions.

JA973676B