A Catalytic Enantioselective Route to Hydroxy-Substituted Quaternary Carbon Centers: Resolution of Tertiary Aldols with a Catalytic Antibody

Benjamin List, Doron Shabat, Guofu Zhong, James M. Turner, Anthony Li, Tommy Bui, James Anderson, Richard A. Lerner,* and Carlos F. Barbas III*

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

Received May 6, 1999

Abstract: Aldolase antibody 38C2-catalyzed resolutions of tertiary aldols were studied. Tertiary aldols proved to be very good substrates for antibody catalyzed retro-aldol reactions. The catalytic proficiency, $(k_{cat}/K_M)/k_{uncat}$, of the antibody for these reactions was on the order of 10^{10} M^{-1}. A fluorogenic tertiary aldol allowed for the quantitative study of enantiomeric excess as a function of reaction conversion, revealing an *E* value of ca. 160 in this case. Study of a variety of substrates demonstrated that antibody-catalyzed retro-aldolization provides rapid entry to highly enantiomerically enriched tertiary aldols, typically >95% ee, containing structurally varied, heteroatom-substituted quaternary carbon centers. The utility of this approach to natural product syntheses has been demonstrated with the syntheses of (+)-frontalin, the side chain of Saframycin H, and formal syntheses of (+)- and (−)-mevalonolactone.

Introduction

The development of strategies for the preparation of enantiomerically pure aldols remains at the frontier of organic synthesis.[1] While the most successful aldol strategies have utilized chiral auxiliaries to direct the stereochemical course of this formidable C−C bond-forming reaction, catalytic strategies involving preformed enolate equivalents have met with encouraging success.[2] Complementing traditional synthetic strategies, families of diverse natural aldolase enzymes have also been enlisted in aldol strategies and have provided many efficient syntheses of carbohydrates and their derivatives.[3] Notably, both chemical and enzymatic approaches have been applied almost exclusively to the synthesis of *secondary β*-hydroxy carbonyl

i) $R^1 = H$, $R^2 = $ alkyl; $\Delta\Delta G^{\ddagger} \approx 1.7$ kcal mol^{-1}
ii) $R^1, R^2 = $ alkyl; $\Delta\Delta G^{\ddagger} \approx 0.1$-$0.5$ kcal mol^{-1}

Figure 1. Zimmerman−Traxler transition-state model for aldehyde−ketone (i) and ketone−ketone (ii) aldol reactions.

compounds (secondary aldols). *General methods, either chemical or enzymatic, for the preparation of enantiomerically enriched tertiary aldols have not been developed.* Tertiary aldols contain a heteroatom-substituted quaternary carbon stereocenter, which constitutes one of the most demanding challenges in synthetic chemistry.[4] This is particularly true when this problem is approached through aldol chemistry. Design of enantioselective small-molecule catalysts for this class of aldols is challenged by the difficulty in achieving diastereotopic transition states that differ significantly in $\Delta\Delta G^{\ddagger}$ to allow for the degree of enantioselectivity desired, approximately 1.8 kcal/mol at −78 °C for an enantiomeric ratio of 99:1. This challenge becomes apparent upon examination of Zimmerman−Traxler transition-state models for this reaction mediated by a small molecule.[5] As shown in Figure 1, in the transition-state model for tertiary aldol reactions, the differential energy between states **A** and **B** when both R^1 and R^2 are aliphatic groups can be estimated to be less than 0.5 kcal/mol for the C_2H_5 through C_6H_{11} series.

(1) For reviews on the aldol reaction, see: Masamune, S.; Choy, W.; Peterson, J. S.; Sita, L. R. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 1. Heathcock, C. H. *Aldrichim. Acta* **1990**, *23*, 99. Evans, D. A. *Science* **1988**, *240*, 420. 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) Nelson, S. G. *Tetrahedron: Asymmetry* **1998**, 357−389. Yanagisawa, A.; Matsumoto, Y.; Nakashima, H.; Asakawa, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1997**, *119*, 9319 and references therein. Carreira, E. M.; Lee, W.; Singer, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 3649. Evans, D. A.; MacMillan, D. W. C.; Campos, K. R. *J. Am. Chem. Soc.* **1997**, *119*, 10859. For a case where ketones are directly used, see: Yamada, Y. M. A.; Yoshikawa, N.; Sasai, H.; Shibasaki, M. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1871. Yoshikawa, N.; Yamada, Y. M. A.; Das, J.; Sasai, H.; Shibasaki, M. *J. Am. Chem. Soc.* **1999**, *121*, 4168−4178.

(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. (c) Henderson, I.; Sharpless, K. B.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 558. (d) 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.; Pergamaon, Oxford, 1991; Vol. 2, p 455. Gijsen, H. J. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 2947. Wong, C.-H.; et al. *J. Am. Chem. Soc.* **1995**, *117*, 3333. Chen, L.; Dumas, D. P.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 741. (e) Fessner, W.-F. *Curr. Opin. Chem. Biol.* **1998**, *2*, 85−97. Fessner, W.-F.; Walter, C. *Top. Curr. Chem.* **1996**, *184*, 97−194.
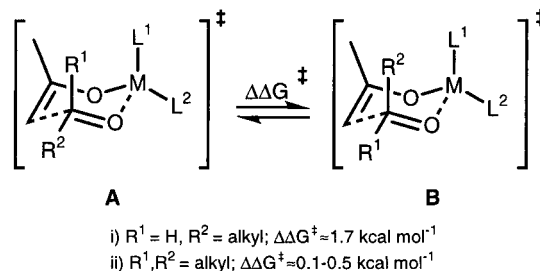
(4) Corey, E. J.; Guzman-Perez, A. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 388−401.

(5) (a) Zimmerman, H. E.; Traxler, M. D. *J. Am. Chem. Soc.* **1952**, *79*, 1920. (b) Denmark, S. E.; Henke, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 2177−2194. (c) Eliel, E. L. *Angew. Chem., Int. Ed. Engl.* **1965**, *14*, 761−774.
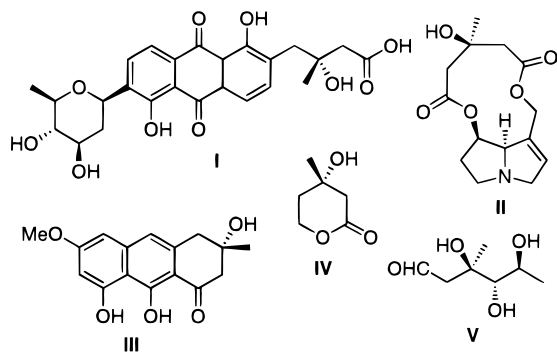
7284   *J. Am. Chem. Soc., Vol. 121, No. 32, 1999*

List et al.

Figure 2. Natural products containing tertiary aldols. (**I**) Vineomycinone B2, (**II**) Dicrotaline, (**III**) Torosachrysone, (**IV**) Mevalonolactone, and (**V**) Mycarose.
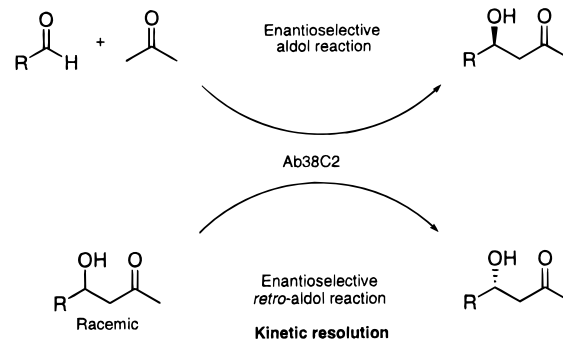
With the standard aldol reaction involving an aldehyde acceptor, however, state **A** is preferred by approximately 1.7 kcal/mol when R^1 is H and R^2 is the least demanding aliphatic group, CH_3. Consequently, only the special case of pyruvate aldols has been solved.[6] Indirect methods for the preparation of enantiomerically enriched tertiary aldols include enantioselective allyl metal additions to ketones, followed by oxidative transformation of the resulting homoallyl alcohols.[7] However, these methods usually give low enantiomeric excesses (ee's), especially when the two alkyl groups differ only slightly. In demanding cases such as these, enzyme catalysis is usually without peer. Through multiple interactions, protein catalysts can accentuate the energy differences in diastereomeric transition states. However, application of natural enzymes to this problem is hindered by the fact that no known natural aldolase catalyzes the synthesis of tertiary aldols.

Tertiary aldols are not only challenging stereochemical problems, but they are also structural motifs common to many bioactive natural products (Figure 2). In this paper, we describe a practical and highly enantioselective route to tertiary aldols that is based on the commercially available aldolase antibody 38C2 (Aldrich Catalog No. 47,995-0). This strategy involves kinetic resolutions of structurally varied tertiary aldols catalyzed by 38C2. Further, we present new UV and fluorescent aldolase assays and convenient methodologies for the preparation of racemic starting materials. The utility of this approach in natural product synthesis is demonstrated.
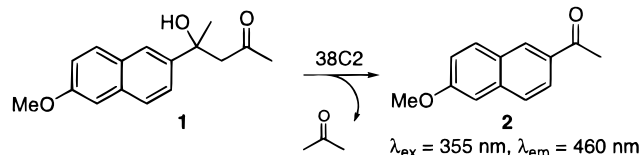
Results and Discussion

We recently described aldolase antibody 38C2 as a highly enantioselective catalyst for the aldol reaction between small, unmodified ketones and a large repertoire of aldehydes.[8] We further demonstrated the use of 38C2 as an efficient catalyst for the retro-aldol reaction, allowing for the kinetic resolution

(6) (a) Kobayashi, S.; Fujishita, Y.; Mukayama, T. *Chem. Lett.* **1989**, 2069−2072. (b) Evans, D. A.; Burgey, C. S.; Kozlowski, M. C.; Tregay, S. W. *J. Am. Chem. Soc.* **1999**, *121*, 686−699 and references therein.

(7) (a) Tietze, F. T.; Schiemann, K.; Wegner, C. *J. Am. Chem. Soc.* **1995**, *117*, 5851−5852. (b) Prabhakar, J.; Bhat, K. S.; Perumal, P. T.; Brown, H. C. *J. Org. Chem.* **1986**, *51*, 432−439. (c) Yasuda, M.; Kitahara, N.; Fujibayashi, T.; Baba, A. *Chem. Lett.* **1998**, 743−744. (d) Riediker, M.; Duthaler, R. O. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 494−495.

(8) (a) Wagner, J.; Lerner, R. A.; Barbas, C. F., III *Science* **1995**, *270*, 1797. (b) Barbas, C. F., III; 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*, 2085−2092. (c) Hoffmann, T.; Zhong, G.; List, B.; Shabat, D.; Anderson, J.; Gramatikova, S.; Lerner, R. A.; Barbas, C. F., III. *J. Am. Chem. Soc.* **1998**, *120*, 2768−2779. (d) List, B.; Shabat, D.; Barbas, C. F., III; Lerner, R. A. *Chem. Eur. J.* **1998**, 881−885.

Scheme 1

Scheme 2. Retro-Aldol Reaction of *tert*-Methodol (**1**)

of racemic secondary aldols.[9] By using both the forward (or synthetic) aldol and the retro-aldol reactions, both aldol enantiomers become accessible (Scheme 1). This is possible because this reaction is characteristically reversible and can be driven to completion in either direction by varying the concentrations of the reactants with respect to the equilibrium constant. In principle, this is true for the case of both secondary and tertiary aldols. For standard aldol reactions between ketone or aldehyde donors (nucleophiles) and aldehyde acceptors (electrophiles) that yield secondary aldols, the equilibrium constant favors the aldol product. For example, the aldol reaction of acetone with benzaldehyde has an equilibrium constant of 12 M^{-1}.[10] Aqueous solutions containing 1 M acetone and 1 mM benzaldehyde reach equilibrium when 92% of benzaldehyde has reacted to form the aldol. Conversely, in the retro-aldol reaction of a 1 mM solution of the aldol, equilibrium is reached at 99% conversion to benzaldehyde. This is in stark contrast to the situation observed with tertiary aldols. In this case, the aldol reaction of acetone with acetophenone has an equilibrium constant of 0.002 M^{-1}.[11] A 1 mM solution of acetophenone would require the concentration of acetone to be 10 000 M (neat acetone, ∼14 M) to reach 95% conversion. However, in the retro-aldol reaction, a 1 mM solution of this tertiary aldol would be converted almost completely to its constituent ketones at equilibrium.

Indeed, we have found antibody 38C2 to be an efficient catalyst for the retro-aldol reaction of tertiary aldols. We have studied this reaction with the highly sensitive fluorogenic aldol sensor *tert*-Methodol (**1**).[12] Treatment of aldol **1** (200 μM) with aldolase antibody 38C2 (2 μM regarding active sites) results in the rapid formation of fluorescent 6′-methoxy-2′-acetonaphthone (**2**), as monitored by fluorescence spectroscopy ($\lambda_{abs} = 355$ nm, $\lambda_{em} = 460$ nm) (Scheme 2). Moreover, we found that the reaction never went beyond 50% conversion, suggesting that the reaction is highly enantioselective (Figure 3). In fact, the

(9) Zhong, G.; Shabat, D.; List, B.; Anderson, J.; Sinha, S. C.; Lerner, R. A.; Barbas, C. F., III. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2481−2484.

(10) Guthrie, J. P.; Cossar, J.; Taylor, K. F. *Can. J. Chem.* **1984**, *62*, 1958.

(11) Guthrie, J. P.; Wang, X.-P. *Can. J. Chem.* **1992**, *70*, 1055−1068.

(12) List, B.; Barbas, C. F., III; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, 15351−15355.
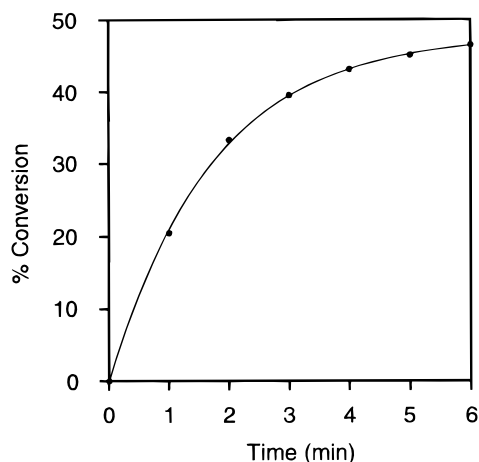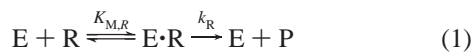
Figure 3. Conversion vs time for the reaction of aldol **1** (5 μM) and 38C2 (1 μM).

ee of the remaining aldol (*R*)-**1** was >99% after 50% conversion, as determined by chiral HPLC analysis (Daicel Chiralcel OD-R).

Quantitative Analysis of Enzymatic Kinetic Resolution. The efficiency of an enzyme-catalyzed kinetic resolution is governed by the two competing reactions between the enzyme (E) and either the R or the S enantiomer to form the product (P) (eqs 1 and 2).

$$E + R \xrightleftharpoons{K_{M,R}} E{\cdot}R \xrightarrow{k_R} E + P \qquad (1)$$

$$E + S \xrightleftharpoons{K_{M,S}} E{\cdot}S \xrightarrow{k_S} E + P \qquad (2)$$

For resolution to be achieved, each reaction must operate with different Michaelis−Menten constants, K_M and/or k_{cat}. Like Sharpless and Sih before us, we have used Kagan's treatment of kinetic resolutions through photodecomposition as a model to quantify the efficiency and selectivity of enzymatic kinetic resolutions.[13]

The concentration of each enantiomer as a function of time (*t*) and the initial concentration (R₀, S₀) at *t* = 0 is given by eqs 3 and 4.

$$[R] = [R_0]\, e^{-(k_R[E]/K_{M,R})t} \qquad (3)$$

$$[S] = [S_0]\, e^{-(k_S[E]/K_{M,S})t} \qquad (4)$$

If one starts with a racemic mixture and adopts the convention that R is the less reactive enantiomer, the enantiomeric excess and conversion C are defined by eqs 5 and 6.

$$ee = \frac{[R] - [S]}{[R] + [S]} = \frac{e^{-(k_R[E]/K_{M,R})t} - e^{-(k_S[E]/K_{M,S})t}}{e^{-(k_R[E]/K_{M,R})t} + e^{-(k_S[E]/K_{M,S})t}} \qquad (5)$$

$$C = 1 - \frac{[R] + [S]}{[R_0] + [S_0]} = 1 - \frac{e^{-(k_R[E]/K_{M,R})t} + e^{-(k_S[E]/K_{M,S})t}}{2} \qquad (6)$$

(13) (a) Balavoine, G.; Moradpour, A.; Kagan, H. B. *J. Am. Chem. Soc.* **1974**, *96*, 5152−5158. (b) Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 6237−6247. (c) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299.

Scheme 3. In an Enzyme-Catalyzed Reaction, the Enantioselectivity (*E*) Is Defined by the Ratio of the Specificity Constants (k_{cat}/K_M) for the Individual Enantiomers
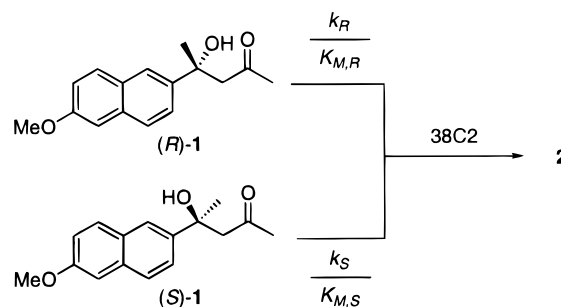
Solving eq 5 for *t* and substituting into eq 6 gives an expression for the relationship between ee and conversion,

$$C = 1 - \frac{1}{2}\left[\left(\frac{1 + ee}{1 - ee}\right)^{1/(1-E)} + \left(\frac{1 + ee}{1 - ee}\right)^{E/(1-E)}\right] \qquad (7)$$

where *E* is defined by the following expression:

$$E = \frac{(k_S/K_{M,S})}{(k_R/K_{M,R})} \qquad (8)$$

Equation 7 is related to Kagan's expression, with the anisotropy factor *g* replaced by an expression in terms of *E* as described by Sih and co-workers. Because *E* is equivalent to the ratio of the specificity constants for the two competing reactions, it is a measure of the degree of discrimination between the two enantiomers and is independent of substrate concentration. Equation 7 allows the relationship between ee and *C* to be graphically represented and the functional dependence on *E* to be observed.

Because *E* is a measure of the degree of an enzyme's discrimination between two enantiomers, we set out to determine the value of *E* for the reaction of Ab38C2 with racemic aldol **1** (Scheme 3).[13c] We measured both ee and conversion simultaneously by HPLC (Figure 4). The data points were fit to eq 7 using a nonlinear least-squares fitting procedure of Kaleida-Graph software (version 3.0.5, Abelbeck software) with *E* as the adjustable parameter. The fit had a correlation coefficient of *R* = 0.998. By this method, we determined the value of *E* for this reaction to be ≥159 ± 50 (Figure 5). For comparison, theoretical plots of ee vs *C* are shown for *E* = 2, 5, 10, and 1000.

Scope and Limitations

Given our encouraging success in the resolution of aldol **1**, we set out to study the range of tertiary aldols that could be resolved using Ab38C2 catalysis. We prepared several racemic tertiary aldols by either of two different paths (A and B, Scheme 4, Table 1). The direct aldol reaction between ketones and an excess of lithium enolates (path A) was successful in many cases. However, aldols prepared from ketones with electonically rich or sterically hindered carbonyl groups were synthesized via allyl metal additions (path B). Subsequent oxidative double bond cleavage of the resulting homoallylic alcohols furnished the desired aldols.[14] We found that the double bond cleavage

(14) Bunelle, W. H.; Rafferty, M. A.; Hodges, S. L. *J. Org. Chem.* **1987**, *52*, 1603−1605. For an alternative strategy, using an allylation/Wacker oxidation sequence, see: Yatagai, H.; Yamamoto, Y.; Maruyama, K. *J. Am. Chem. Soc.* **1980**, *102*, 4548−4550.
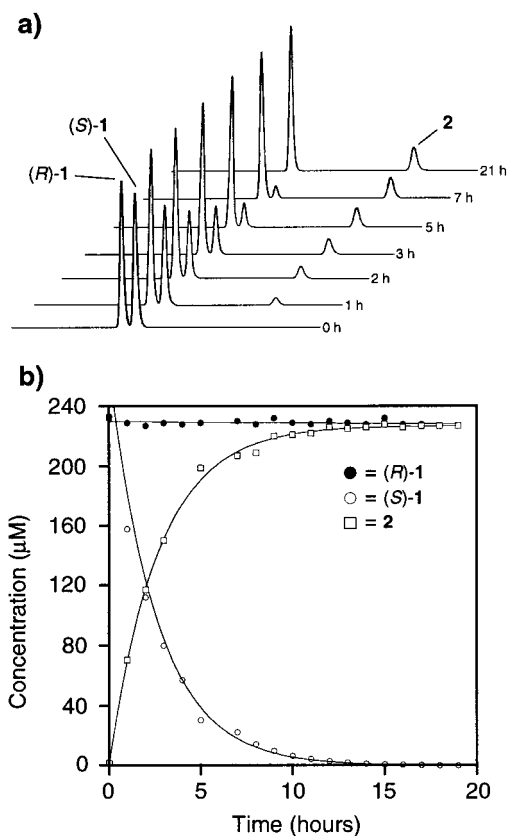
a)

b)

Figure 4. Monitoring the retro-aldol kinetic resolution of aldol **1** by chiral HPLC (480 μM aldol **1**, 2 μM 38C2). (a) Actual (unmodified) HPLC chromatograms for the reaction at different time points. (b) Plot of the concentration of (*R*)-**1**, (*S*)-**1**, and **2** vs time.
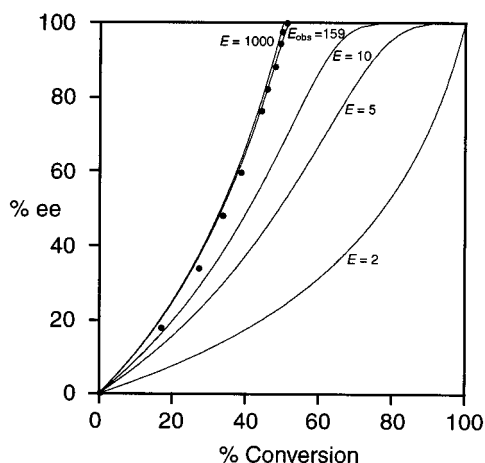

Figure 5. Interdependence of conversion and ee for the resolution of **1** as monitored by HPLC. For comparison, theoretical curves are shown for $E = 2$, 5, 10, and 1000. The measured data points are indicated as filled circles (●).

was most successful using a two-step, one-pot sequence of dihydroxylation (OsO$_4$, NMO) and lead tetraacetate cleavage.

The racemic tertiary aldols were then subjected to kinetic resolution with Ab38C2. Chromogenic aldols **5** and **6** were designed to allow for sensitive continuous UV assays since retro-aldolization provides 4-methoxy-styrylketones ($\lambda_{max} \approx 315$). The results of kinetic resolutions with antibody 38C2 are shown in Table 2. *The ee's of the products as determined by chiral-phase HPLC analysis (Table 3) were usually ≥95% after 50% conversion.* The data presented in Table 2 demonstrate that catalytic enantioselective retro-aldol reactions can provide access

Scheme 4. Two Methods Used for the Synthesis of Racemic Tertiary Aldols Used in This Study
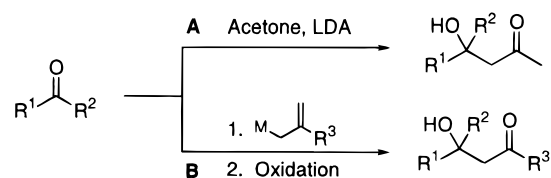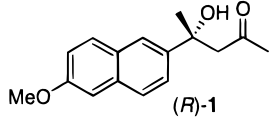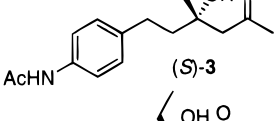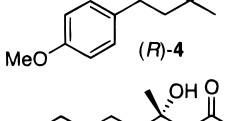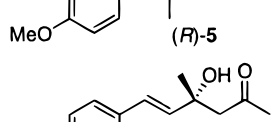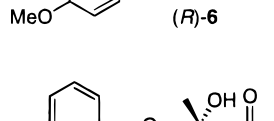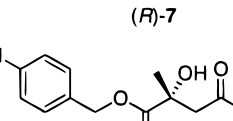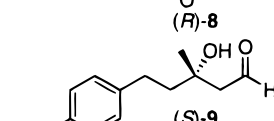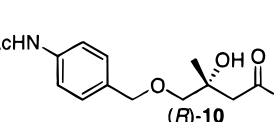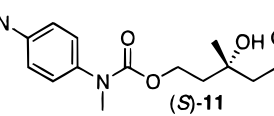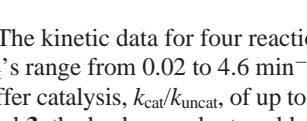

Table 1. Tertiary Aldols Studied

Aldol	Method	Yield
1	B[12]	75%[a]
3	A	87%
4	A	34%
5	A	40%
6	A	83%
7	A	78%
8	A	85%
9	B	71%[b]
10	B	74%[a]
11	--[c]	--[c]

[a] Over three steps. [b] Over two steps. [c] See Experimental Section.

to a variety of hydroxy-substituted quaternary carbon stereo-centers with excellent ee's. Further, both ketone and aldehyde aldols are readily resolved. The kinetic resolution of aldols **9**–**11** demonstrates the potential of aldehyde aldols. Aldehyde aldols provide facile access to acetate aldols that are otherwise difficult to obtain by more traditional techniques.[15]

Table 2. Tertiary Aldols Obtained via Kinetic Resolution with 38C2

Product	Conversion	ee
(R)-1	50%	>99%
(S)-3	52%	80%
(R)-4	50%	>99%
(R)-5	50%	94%
(R)-6	50%	96%
(R)-7	50%	96%
(R)-8	50%	95%
(S)-9	40%	75%
(R)-10	50%	95%
(S)-11	50%	95%

Table 3. Conditions for the Separation of Aldol Enantiomers Using Chiral HPLC

aldol	HPLC conditions (Chiracel column, λ, flow rate, solvent)	retention times (min)[b]
1	OD-R, 229 nm, 0.8 mL/min, 35% CH_3CN/H_2O (0.1% TFA)	t_R = 28.2 t_S = 30.6
3	AD, 254 nm, 1.0 mL/min, 20% IPA/hexane	t_R = 21.2 t_S = 23.9
4	AS, 254 nm, 1.0 mL/min, 8% IPA/hexane	t_R = 8.4 t_S = 11.0
5	AS, 254 nm, 1.0 mL/min, 8% IPA/hexane	t_R = 9.4 t_S = 14.9
6	AS, 254 nm, 1.0 mL/min, 8% IPA/hexane	t_R = 20.4 t_S = 15.1
7	OD-R, 254 nm, 0.8 mL/min, 20% CH_3CN/H_2O (0.1% TFA)	t_R = 25.1 t_S = 26.2
8	OG, 254 nm, 1.0 mL/min, 20% IPA/hexane	t_R = 14.2 t_S = 18.1
10[a]	OD-R, 254 nm, 0.8 mL/min, 15% CH_3CN/H_2O (0.1% TFA)	t_R = 15.3 t_S = 16.8
11[a]	AD, 307 nm, 1.0 mL/min, 14% IPA/hexane	t_R = 27.8 t_S = 23.2

[a] After reduction to the alcohol ($NaBH_4$, MeOH). [b] In t_R/t_S, R and S refer to the absolute configurations of the aldols.

Table 4. Kinetic Parameters for Selected Retro-Aldol Reactions

substrate	k_{cat} (min^{-1})	K_M (mM)	k_{cat}/k_{uncat}	$(k_{cat}/K_M)/k_{uncat}$ (M^{-1})
1	1.8	0.12[a]	1.2×10^6	1.3×10^{10}
5	4.6	0.11[b]	8.4×10^5	7.7×10^9
3	0.15	1.62[b]	nd[c]	nd
11	0.02	0.13[b]	nd	nd

[a] K_M for the substrate enantiomer (S)-1. [b] K_M for the racemic mixture. [c] nd = not determined.

(R)-enantiomer proved to be a potent competetive inhibitor. To obtain a detailed kinetic analysis, the inhibitor aldol (R)-1 was prepared on a preparative scale (50 mg) by an antibody 38C2-catalyzed kinetic resolution.[16] We measured the rates at different substrate ((S)-1) and inhibitor ((R)-1) concentrations according to eq 9.[17] Dixon plot analysis (Figure 6) provided K_i = 92 $\pm$

$$\frac{1}{\nu} = \frac{K_M}{k_{cat}[S]K_i}[I] + \frac{1}{k_{cat}}\left(1 + \frac{K_M}{[S]}\right) \qquad (9)$$

11 μM for aldol (R)-1. Plotting the different slopes of the Dixon plot versus the inverse substrate concentrations provided K_M = 120 $\pm$ 37 μM for (S)-1. The result that both enantiomers of 1 bind to Ab38C2 with similar affinities ($K_i \approx K_M$) rules out the possibility that enantiomer discrimination is due to preferential binding of one enantiomer.

Application to Natural Product Synthesis. Initially, the absolute configuration of the products was tentatively assigned on the basis of our results with secondary aldols. Further evidence was provided by a rapid synthesis of the pheromone (+)-frontalin (**13**), which was first synthesized by Mori and co-workers and is a sex pheromone found in several beetle species[18] and, surprisingly, in the temporal gland secretion of the male Asian elephant.[19]

Lithium hydroxide-mediated Horner–Wadsworth–Emmons reaction[20] of aldehyde (R)-10 with diethyl (2-oxopropyl)-

The kinetic data for four reactions are shown in Table 4. The k_{cat}'s range from 0.02 to 4.6 min^{-1} with rate enhancements over buffer catalysis, k_{cat}/k_{uncat}, of up to 10^6. For most cases, including aldol **3**, the background retro-aldolization reaction was undetectable at a substrate concentration of 5 mM, even after a week. The catalytic proficiency, $(k_{cat}/K_M)/k_{uncat}$, for substrates **1** and **5** is $\sim10^{10}$ M^{-1}. Antibody 38C2 has been shown to be the most proficient catalytic antibody described to date, and this proficiency is maintained with the tertiary aldol substrates described here.

Enantiomeric Discrimination Is Not Achieved through Preferential Binding. While aldol (S)-1 is an efficient substrate (k_{cat} = 1.8 min^{-1}, k_{cat}/k_{uncat} = 1.2 $\times$ 10^6), the corresponding

(15) Saito, S.; Hatanaka, K.; Kano, T.; Yamamoto, H. *Angew. Chem., Int. Ed.* **1999**, *37*, 3378−3381 and references therein.

(16) To be published.
(17) Segel, I. H. *Enzyme Kinetics*; John Wiley & Sons: New York, 1975; pp 109−111.
(18) Mori, K.; Nishimura, Y. *Eur. J. Org. Chem.* **1998**, *233*, 3 and references therein.
(19) Perrin, T. E.; Rasmussen, L. E. L.; Gunawardena, R.; Rasmussen, R. A. *J. Chem. Ecol.* **1996**, *22*, 207.
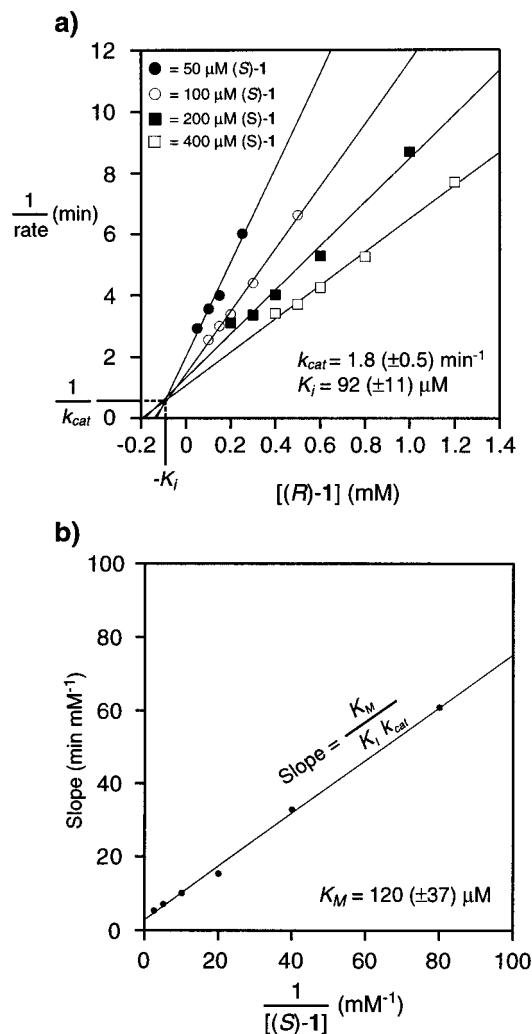(20) Mulzer, J.; List, B. *Tetrahedron Lett.* **1994**, *35*, 9021−9024.

a)

b)

Figure 6. (a) Dixon plot of 1/rate vs inhibitor enantiomer (*R*)-**1** concentration in the presence of different fixed concentrations of substrate enantiomer (*S*)-**1**. (b) Replot of the slopes of the Dixon plot vs 1/substrate enantiomer (*S*)-**1** concentration.
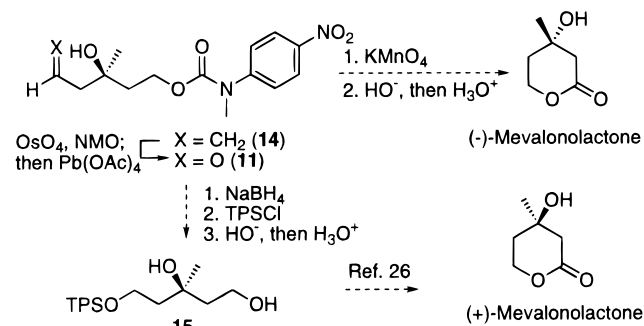
Scheme 5. Enantioselective Synthesis of (+)-Frontalin via Kinetic Resolution with 38C2
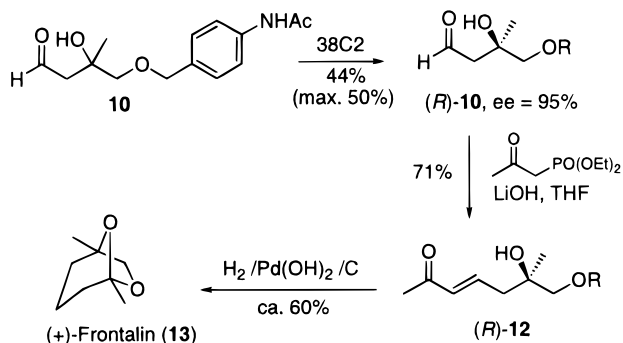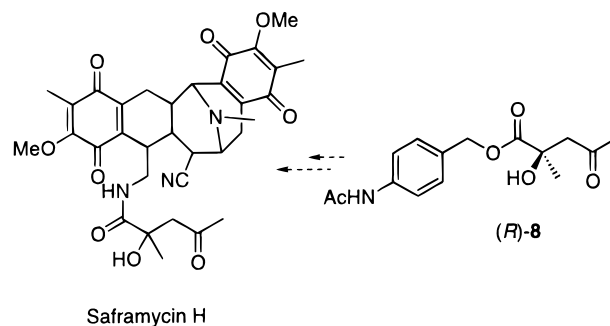

phosphonate gave the α,β-unsaturated ketone (*R*)-**12**. Hydrogenolysis provided (+)-frontalin (**13**) ($[\alpha]^{23}_D$ +48.3, lit.[21] $[\alpha]^{23}_D$ +54.4), which unambiguously proved the assigned stereochemistry (Scheme 5).

We further developed formal syntheses of (−)- and (+)-mevalonolactone (Scheme 6).[22] Oxidation of racemic aldehyde

(21) Ohrui, H.; Emoto, S. *Agric. Biol. Chem.* **1976**, *40*, 2267.
(22) Wolf, D. E.; Hoffmann, C. H.; Aldrich, P. E.; Skegys, H. R.; Wright, L. D.; Folkers, K. *J. Am. Chem. Soc.* **1956**, *78*, 4499.

Scheme 6. Formal Syntheses of (+)- and (−)-Mevalonolactone

Scheme 7

Saframycin H

11 with KMnO₄,[23] followed by deprotection of the carbamate under basic conditions,[24] and acid-catalyzed cyclization furnished (±)-mevalonolactone. Since these conditions are known not to cause extensive racemization,[24] we conclude that aldehyde (*S*)-**11** could be converted to (−)-mevalonolactone. Known diol (*S*)-**15** (which we obtained in racemic form via reduction, silyl protection, and carbamate hydrolysis[25] of racemic aldehyde **11**) has recently been converted to (+)-mevalonolactone.[26]

The utility of the antibody-catalyzed kinetic resolution of tertiary aldols in natural product synthesis was further demonstrated by the first enantioselective preparation of the Saframycin H side chain (Scheme 7). While other members of the antitumor-active Saframycin family have been synthesized,[27] Saframycin H remains an elusive goal for synthetic organic chemists.[28] Furthermore, the absolute configuration of the tertiary aldol stereocenter of the side chain is unknown. In this case, kinetic resolution of aldol **8** provides (*R*)-**8** with 95% ee at 50% conversion. Compound (*R*)-**8** could help to prove the absolute configuration of the Saframycin H side chain and may be a useful starting material for the synthesis of Saframycin H.

Conclusions

We have demonstrated that antibody-catalyzed retro-aldolization of tertiary aldols provides a rapid entry to structurally varied and highly enantiomerically enriched tertiary aldols. This result highlights the potential synthetic utility of catalytic antibodies as artificial enzymes in addressing problems in organic chem-

(23) Abiko, A.; Roberts, J. C.; Takemasa, T.; Masamune, S. *Tetrahedron Lett.* **1986**, *27*, 4537−4540.
(24) Mori, K.; Okada, K. *Tetrahedron* **1985**, *41*, 557−559.
(25) Gassman, P. G.; Hodgson, P. K. G.; Balchunis, R. J. *J. Am. Chem. Soc.* **1976**, *98*, 1275−1276.
(26) Krohn, K.; Meyer, A. *Liebigs Ann. Chem.* **1994**, 167−174.
(27) (a) Kishi, K.; Yazawa, K.; Takahashi, K.; Mikami, Y.; Arai, T. *J. Antibiot.* **1984**, *37*, 847. (b) Mikami, Y.; Takahashi, K.; Yazawa, K.; Hour-Young, C.; Arai, T. *J. Antibiot.* **1988**, *41*, 734.
(28) Fukuyama, T.; Yang, L.; Ajeck, K. L.; Sachleben, R. A. *J. Am. Chem. Soc.* **1990**, *112*, 3712.

istry that are not solved by natural enzymes or more traditional synthetic methods. The continuous UV and fluorescent assays developed here for the study of the retro-aldolization of tertiary aldols should be useful tools for the discovery of additional catalysts of this reaction. These assays are compatible with high-throughput combinatorial approaches to asymmetric catalysts. As we have demonstrated here with the synthesis of (+)-frontalin and the side chain of Saframycin H, and formal syntheses of (+)- and (−)-mevalonolactone, this approach should provide access to a variety of enantiomerically enriched synthons that may be applied to natural product syntheses.

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. For thin-layer chromatography (TLC), Merck 60 F_{254} silica gel plates were used, and 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 concentrated 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 concentrated 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. For flash chromatography (FC), Merck 60 silica gel (particle size 0.040−0.063 mm) was used; the eluent is given in parentheses. For ^1H NMR, Bruker DRX 500, AMX 400, AM 300, and AC 250 instruments were used. The chemical shifts are given in δ relative to TMS ($\delta = 0$ ppm), and the coupling constants J are given in hertz. The spectra were recorded in $CDCl_3$ as solvent at room temperature unless stated otherwise. For HR-MS, liquid secondary ionization (LSI-MS) used a VG ZAB-ZSE with 3-nitrobenzyl alcohol matrix.

Antibody-Catalyzed Resolutions. All antibody-catalyzed reactions were performed in PBS (0.01 M phosphate buffer, 2.7 mM KCl, 137 mM NaCl, pH 7.4). Antibody-catalyzed reactions and background reactions 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 1.0 mL/min. For the retro-aldol reaction of aldol **1**, the formation of ketone **2** was followed by fluorescence spectroscopy ($\lambda_{abs} = 355$ nm, $\lambda_{em} = 460$ nm).

Determination of Enantiomeric Excess of Products. To a 6.25 mM solution of the aldol substrate in 160 μL of PBS was added 20−40 μL of a 100 μM solution of the antibody in PBS. The reaction was diluted with PBS as necessary to bring the volume to 200 μL. The final concentrations were 5 mM of the aldol substrate and 10−20 μM of antibody in a total volume of 200 μL of PBS. After a certain completion of the reaction was reached, the remaining aldol was isolated by reversed-phase HPLC. The residue was redissolved in ca. 200 μL of 2-propanol, and the ee was determined by normal-phase HPLC using an appropriate Daicel column for enantiomer separation.

Synthesis of Racemic Tertiary Aldols. Path A: Direct Cross Aldolization of Two Ketones with LDA. LDA solution (Aldrich, 2 M, heptane/THF/ethylbenzene, 2.5 mL, 5 mmol) was added dropwise to a stirred solution of acetone (0.36 mL, 5 mmol) in anhydrous THF at −78 °C. After 30 min, the second ketone (0.5 mmol) was added, and stirring was continued for another 30 min. The reaction was then quenched with saturated ammonium chloride and was extracted with ether. The organic layer was washed with brine, dried (Na_2SO_4), filtered, concentrated, and purified by column chromatography on silica gel (ethyl acetate/hexane mixtures) to give the pure aldols.

***N*-[4-(3-Hydroxy-3-methyl-5-oxohexyl)phenyl]acetamide (3).** Aldol **3** (87%) was synthesized from *N*-[4-(3-oxobutyl)phenylacetamide[29] as described in the general procedure for path A.

---

(29) Björnstedt, R.; Zhong, G.; Lerner, R. A.; Barbas, C. F., III. *J. Am. Chem. Soc.* **1996**, *118*, 11720−11724.

^1H NMR (300 MHz, $CDCl_3$): δ 7.37 (d, $J = 8.4$ Hz, 2H), 7.36 (br, 1H), 7.09 (d, $J = 8.4$ Hz, 2H), 3.89 (br, 1H), 2.61 (m, 4H), 2.14 (s, 3H), 2.13 (s, 3H), 1.75 (m, 2H), 1.25 (s, 3H). HRMS (MH$^+$): calcd for $C_{15}H_{22}NO_3$, 264.1600; obsd, 264.1607.

4-Hydroxy-4-(4-methoxyphenyl)hexan-2-one (4). Aldol **4** (40%) was synthesized from 1-(4-methoxyphenyl)propan-1-one as described in the general procedure for path A.

^1H NMR (300 MHz, $CDCl_3$): δ 7.29 (d, $J = 8.8$ Hz, 2H), 6.86 (d, $J = 8.8$ Hz, 2H), 4.45 (s, 1H), 3.80 (s, 3H), 3.18 (d, $J = 16.8$ Hz, 1H), 2.80 (d, $J = 16.8$ Hz, 1H), 2.06 (s, 3H), 1.75 (m, 2H), 0.75 (t, $J = 7.3$ Hz, 3H).

4-Hydroxy-6-(4-methoxyphenyl)-4,5-dimethylhex-5-en-2-one (5). Aldol **5** (34%) was synthesized from 4-(4-methoxyphenyl)-3-methylbut-3-en-2-one[30] as described in the general procedure for path A.

^1H NMR (250 MHz, $CDCl_3$): δ 7.27 (d, $J = 8.7$ Hz, 2H), 6.82 (d, $J = 8.7$ Hz, 2H), 6.52 (s, 1H), 4.13 (s, 1H), 3.78 (s, 3H), 3.01 (d, $J = 16.9$ Hz, 1H), 2.75 (d, $J = 16.9$ Hz, 1H), 2.16 (s, 3H), 1.37 (s, 3H).

4-Hydroxy-6-(4-methoxyphenyl)-4-methylhex-5-en-2-one (6). Aldol **6** (83%) was synthesized from 4-(4-methoxyphenyl)but-3-en-2-one as described in the general procedure for path A.

^1H NMR (250 MHz, $CDCl_3$): δ 7.27 (d, $J = 8.7$ Hz, 2H), 6.82 (d, $J = 8.7$ Hz, 2H), 6.52 (d, $J = 16.0$ Hz, 1H), 6.10 (d, $J = 16.0$ Hz, 1H), 4.13 (s, 1H), 3.78 (s, 3H), 2.84 (d, $J = 16.9$ Hz, 1H), 2.69 (d, $J = 16.9$ Hz, 1H), 2.16 (s, 3H), 1.37 (s, 3H). HRMS (FAB) (MNa$^+$): calcd for $C_{14}H_{19}O_3Na$, 257.1154; obsd, 257.1159.

5-Benzyloxy-4-hydroxy-4-methylpentan-2-one (7). Aldol **7** (78%) was synthesized from 1-benzyloxypropan-2-one[31] as described in the general procedure for path A.

^1H NMR (400 MHz, $CDCl_3$): δ 7.32 (m, 5H), 4.52 (s, 2H), 3.83 (s, 1H), 3.39 (d, $J = 9.1$ Hz, 1H), 3.33 (d, $J = 9.1$ Hz, 1H), 2.83 (d, $J = 16.5$ Hz, 1H), 2.53 (d, $J = 16.5$ Hz, 1H), 2.15 (s, 3H), 1.23 (s, 3H). HRMS (FAB) (MH$^+$): calcd for $C_{13}H_{19}O_3$, 223.1344; obsd, 223.1328.

2-Hydroxy-2-methyl-4-oxopentanoic Acid 4-Acetylaminobenzyl Ester (8). Pyruvoyl chloride (746 mg, 7.0 mmol) was added dropwise to a stirred solution of *N*-(4-hydroxymethylphenyl)acetamide (1.0 g, 6.0 mmol) and triethylamine (1.0 mL, 10 mmol) in 30 mL of methylene chloride at 0 °C. After 30 min, the mixture was worked up with water and methylene chloride. The organic layer was dried (Na_2SO_4), filtered, concentrated, and purified by column chromatography on silica gel (40% ethyl acetate/hexane) to give 2-oxopropionic acid 4-acetyl-aminobenzyl ester (1.28 g, 91%).

^1H NMR (400 MHz, $CDCl_3$): δ 7.51 (d, $J = 8.4$ Hz, 2H), 7.41 (br, 1H), 7.32 (d, 8.4H), 5.21 (s, 2H), 2.46 (s, 3H), 2.16 (s, 3H).

Aldol **8** (85%) was synthesized from 2-oxopropionic acid 4-acetyl-aminobenzyl ester as described in the general procedure for path A.

^1H NMR (500 MHz, $CDCl_3$): δ 7.47 (d, $J = 8.0$ Hz, 2H), 7.36 (br, 1H), 7.25 (d, $J = 8.0$ Hz, 2H), 5.11 (s, 2H), 3.09 (d, $J = 17.5$ Hz, 1H), 2.77 (d, $J = 17.5$ Hz, 1H), 2.15 (s, 3H), 2.09 (s, 3H), 1.35 (s, 3H). HRMS (FAB) (MH$^+$): calcd for $C_{15}H_{20}NO_5$, 294.1341; obsd, 294.1349.

***N*-[4-(3-Hydroxy-3-methyl-5-oxopentyl)phenyl]acetamide (9).** Allyl magnesium bromide (1.0 M, 1.5 mL, 1.5 mmol) was added dropwise to a stirred solution of *N*-[4-(3-oxobutyl)phenyl]acetamide (100 mg, 0.5 mmol) in anhydrous THF at −78 °C. After 30 min, the reaction was quenched with saturated ammonium chloride and extracted with ether. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated in vacuo to give *N*-[4-(3-hydroxy-3-methylhex-5-enyl)phenyl]acetamide (112 mg, 91%).

^1H NMR (400 MHz, $CDCl_3$): δ 7.38 (d, $J = 8.3$ Hz, 2H), 7.20 (br, 1H), 7.13 (d, $J = 8.3$ Hz, 2H), 5.86 (m, 1H), 5.15 (m, 2H), 2.65 (m, 2H), 2.28 (d, $J = 7.4$ Hz, 2H), 1.73 (m, 2H), 1.23 (s, 3H).

N-[4-(3-Hydroxy-3-methylhex-5-enyl)phenyl]acetamide (110 mg, 0.45 mmol) was ozonized in methanol at −78 °C until the blue color of ozone was observed. Then, the excess of ozone was removed by bubbling oxygen through the solution, and the ozonide was quenched with dimethyl sulfide. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography on silica gel (80% ethyl acetate/hexane) to give aldol **9** (89 mg, 78%).

---

(30) Kawai, Y.; Saitou, Y.; Kentarou, H.; Kouchi, H.; Dao, D. H.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2633−2638.

(31) Hon, Y. S.; Lin, S. W.; Chen, Y. J.; Hon, Y. S.; Lin, S. W.; Chen, Y. J. *Synth. Commun.* **1993**, *23*, 1543−1553.

^{1}H NMR (400 MHz, CDCl$_3$): δ 9.86 (t, J = 1.8 Hz, 1H), 7.38 (d, J = 8.5 Hz, 2H), 7.35 (br, 1H), 7.10 (d, J = 8.5 Hz, 2H), 2.65 (m, 4H), 2.14 (s, 3H), 1.82 (m, 2H), 1.34 (s, 3H).

N-[4-(2-Hydroxy-2-methyl-4-oxobutoxymethyl)phenyl]acetamide (10). Sodium hydride (480 mg, 12.0 mmol) was added to a stirred solution of 2-methyl-2-propen-1-ol (925 μL, 11.0 mmol) in 10 mL of DMF at 0 °C. After 10 min, N-(4-chloromethylphenyl)acetamide (1.0 g, 5.4 mmol) was added, and the reaction mixture was stirred for 1 h. Then, the mixture was worked up with water and ether. The organic layer was washed with brine and dried (Na$_2$SO$_4$), and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (40% ethyl acetate/hexane) to give N-[4-(2-methylallyloxymethyl)phenyl]acetamide (670 mg, 56%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 7.48 (d, J = 8.5 Hz, 2H), 7.26 (d, J = 8.5 Hz, 2H), 7.21 (br, 1H), 4.95 (d, J = 20.4 Hz, 2H), 4.46 (s, 2H), 3.90 (s, 2H), 2.16 (s, 3H), 1.78 (s, 3H).

To a stirred solution of N-[4-(2-methylallyloxymethyl)phenyl]acetamide (657 mg, 3.0 mmol) in 10 mL of acetone was added 4-methylmorpholine N-oxide (50% solution in water, 684 μL, 3.3 mmol), followed by osmium tetraoxide (2.5% solution in 2-methyl-2-propanol, 1.25 mL, 0.1 mmol). The reaction mixture was stirred for 1 h. After it was confirmed that no more starting material was left according to TLC, sodium periodate (1.28 g, 6.0 mmol) was added, and the mixture was stirred for another hour. Then it was worked up with water and methylene chloride. The organic phase was dried (Na$_2$SO$_4$), and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (50% ethyl acetate/hexane) to give N-[4-(2-oxopropoxymethyl)phenyl]acetamide (625 mg, 94%).

^{1}H NMR (400 MHz, CDCl$_3$): δ 7.48 (d, J = 8.5 Hz, 2H), 7.30 (br, 1H), 7.26 (d, J = 8.5 Hz, 2H), 4.53 (s, 2H), 4.03 (s, 2H), 2.16 (s, 3H), 2.14 (s, 3H).

To a stirred solution of N-[4-(2-oxopropoxymethyl)phenyl]acetamide (625 mg, 2.8 mmol) in 10 mL of methylene chloride was added scandium trifluoromethanesulfonate (100 mg, 0.2 mmol), followed by tetraallyl tin (340 μL, 1.5 mmol), and the mixture was stirred for 1 h. Then it was worked up with water and methylene chloride. The organic layer was dried (Na$_2$SO$_4$), and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (50% ethyl acetate/hexane) to give N-[4-(2-hydroxy-2-methylpent-4-enyloxymethyl)phenyl]acetamide (605 mg, 82%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 7.47 (d, J = 8.5 Hz, 2H), 7.45 (br, 1H), 7.25 (d, J = 8.5 Hz, 2H), 6.81 (m, 1H), 5.07 (m, 2H), 4.49 (s, 2H), 3.30 (d, J = 9.0 Hz, 1H), 3.25 (d, J = 9.0 Hz, 1H), 2.33 (s, 1H), 2.25 (m, 2H), 2.16 (s, 3H), 1.16 (s, 3H).

Aldol **10** (90%) was prepared from N-[4-(2-hydroxy-2-methylpent-4-enyloxymethyl)phenyl]acetamide via dihydroxylation/periodate cleavage as described in the synthesis of N-[4-(2-oxopropoxymethyl)phenyl]acetamide.

^{1}H NMR (400 MHz, CDCl$_3$): δ 9.83 (t, J = 2.4 Hz, 1H), 7.47 (d, J = 8.5 Hz, 2H), 7.33 (br, 1H), 7.24 (d, J = 8.5 Hz, 2H), 4.49 (s, 2H), 3.34 (s, 2H), 2.91 (s, 1H), 2.68 (d, J = 18.2 Hz, 1H), 2.49 (d, J = 18.2 Hz, 1H), 2.19 (s, 3H), 1.25 (s, 3H).

(+)-Frontalin (13). Racemic aldol **10** (50 mg, 0.19 mmol) was incubated with antibody 38C2 (100 mg, 0.00067 mmol) in 50 mL of PBS (pH 7.4). The reaction was followed by HPLC, and after 7 days (k_{cat} = 0.0034 min^{-1}) it reached 50% conversion and stopped. The reaction mixture was saturated with NaCl and extracted with ethyl acetate. The organic layer was dried (Na$_2$SO$_4$), and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (70% ethyl acetate/hexane) to give aldol (R)-**10** (22 mg, 44%) in 95% ee.

To a stirred solution of aldol (R)-**10** (22 mg, 0.083 mmol) in 2 mL of THF was added diethyl (2-oxopropyl)phosphonate (48 mg, 0.25 mmol), followed by lithium hydroxide monohydrate (6.0 mg, 0.25 mmol). The reaction mixture was stirred for 2 h. Then it was worked up with water and ethyl acetate. The organic layer was dried (Na$_2$SO$_4$), filtered, concentrated in vacuo, and purified by column chromatography on silica gel (70% ethyl acetate/hexane) to give enone **12** (18 mg, 71%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 8.26 (br, 1H), 7.46 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 8.5 Hz, 2H), 6.77 (m, 1H), 5.99 (d, J = 16.0 Hz, 1H), 4.43 (s, 2H), 3.25 (s, 2H), 2.42 (m, 1H), 2.31 (m, 1H), 2.15 (s, 3H), 2.09 (s, 3H), 1.11 (s, 3H).

Compound **12** (18 mg, 0.059 mmol) was hydrogenated with a catalytic amount of Pd(OH)$_2$/C in methanol for 2 h. The reaction mixture was filtered through Celite, and the solvent was removed under reduced pressure to give a 12-mg mixture of (+)-Frontalin along with p-acetamidotoluene, [α]^{23}$_D$ +48.3 (lit.[13] [α]^{23}$_D$ +54.4). Due to the small amount and the volatile nature of Frontalin (**13**), we were unable to purify it to get an accurate reading of the optical rotation.

(4-Nitrophenyl)methylcarbamic Acid 3-Hydroxy-3-methyl-5-oxopentyl Ester (11). To 4-hydroxy-2-butanone (2 mL, 23.2 mmol) and Sc(OTf)$_3$ (570 mg, 1.3 mmol) in CH$_2$Cl$_2$ (8 mL) was added tetraallyltin (2.8 mL, 11.6 mmol). The mixture was stirred for 16 h at room temperature. After evaporation and column chromatography (50−66% ethyl acetate/hexane), 3-methyl-5-hexene-1,3-diol[32] was isolated (2.6 g, 20 mmol, 86%).

^{1}H NMR (300 MHz, CDCl$_3$): δ 5.75−5.85 (m, 1H), 5.15 (m, 2H), 3.88 (br, 2H), 3.49 (br, 1H), 3.19 (br, 1H), 2.25 (d, J = 7.4 Hz, 2H), 1.60−1.82 (m, 2H), 1.19 (s, 3H).

To a stirred solution of phosgene (0.7 mL, 1.93 M toluene, 1.4 mmol) in 6 mL of dry THF was added a mixture of N-methyl-4-nitroaniline (200 mg, 1.3 mmol) and triethylamine (0.18 mL, 1.3 mmol) in 3 mL of dry THF. The mixture was stirred for 15 min at 0 °C under argon and was warmed to room temperature. To this mixture were added at room temperature 3-methyl-5-hexene-1,3-diol (170 mg, 1.3 mmol), triethylamine (0.18 mL, 1.3 mmol), and DMAP (15 mg) in 2 mL of dry THF. The mixture was stirred at room temperature under argon for 90 h and was worked up with ether/saturated aqueous ammonium chloride. The organic layer was dried (Na$_2$SO$_4$), filtered, concentrated in vacuo, and purified by column chromatography (33% ethyl acetate/hexane) to give carbamate **14** (285 mg, 0.93 mmol, 71%).

^{1}H NMR (300 MHz, CDCl$_3$): δ 8.22 (m, 2H), 7.49 (m, 2H), 5.75−5.89 (m, 1H), 5.18 (m, 2H), 4.38 (m, 2H), 3.40 (s, 3H), 2.25 (m, 2H), 1.64−1.89 (m, 2H), 1.21 (s, 3H). ^{13}C NMR (300 MHz, CDCl$_3$): δ 221, 155, 148.5, 133, 124.5, 124, 119, 71, 63, 47, 40.5, 37, 26. HRMS (FAB): M + H$^+$ 309.1450 (expected), 309.1456 (observed).

To a solution of carbamate **14** (471.2 mg, 1.5 mmol) in 9 mL of dry methylene chloride were added OsO$_4$ (2.5% solution in 2-methyl-2-propanol, 0.8 mL) and NMO (50% aqueous, 0.38 mL, 1.8 mmol). The resulting mixture was stirred for 45 min, and then Pb(OAc)$_4$ was added. After 10 min, it was filtered over Celite, evaporated in vacuo, and purified by column chromatography on silica gel using hexane/ethyl acetate (1:1) to afford aldehyde **11** (409.1 mg, 1.3 mmol, 90%).

^{1}H NMR (300 MHz, CDCl$_3$): δ 9.82 (s, 1H), 8.23 (m, 2H), 7.49 (m, 2H), 4.42 (m, 2H), 3.40 (s, 3H), 2.73 (m, 2H), 1.90−2.10 (m, 2H), 1.37 (s, 3H). ^{13}C NMR (300 MHz, CDCl$_3$): δ 222, 220, 162, 142, 134, 125, 124, 71, 63, 53.5, 41, 37.5, 27.5, 20. MS (FAB): M + Na$^+$ 333 (expected), 333 (observed).

Mevalonolactone. To a solution of aldehyde **11** (340 mg, 1.1 mmol) in 4 mL of anhydrous MeOH was added NaBH$_4$ (46 mg, 1.2 mmol) at 0 °C under argon. The mixture was stirred at room temperature for 30 min and worked up with ether/saturated ammonium chloride. The organic layer was dried (Na$_2$SO$_4$), filtered, concentrated in vacuo, and purified by column chromatography (75% ethyl acetate/hexane) to yield (4-nitrophenyl)methylcarbamic acid 3,5-dihydroxy-3-methylpentyl ester (241 mg, 0.77 mmol, 70%).

^{1}H NMR (300 MHz, CDCl$_3$): δ 8.25 (m, 2H), 7.49 (m, 2H), 4.41 (m, 2H), 4.05−4.20 (m, 2H), 3.91 (br, 2H), 3.40 (s, 3H), 1.60−2.10 (m, 4H), 1.30 (s, 3H). ^{13}C NMR (300 MHz, CDCl$_3$): δ 224, 129, 125, 124, 123.5, 119, 73, 59.5, 44, 41.5, 35, 28, 20. MS (FAB): M + Na$^+$ 335 (expected), 335 (observed).

(4-Nitrophenyl)methylcarbamic acid 3,5-dihydroxy-3-methylpentyl ester (46.3 mg, 0.15 mmol), TPSCl (80 μL, 0.29 mmol), and imidazole (31 mg, 0.46 mmol) were stirred in DMF (2 mL) at room temperature for 24 h. The reaction was then partitioned between ether and 10% HCl. The organic layer was washed with saturated NaHCO$_3$ and water,

(32) Ferraboschi, P.; Caevotti, R.; Grisenti, P.; Santaniello, E. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2301−2303.

dried (Na_2SO_4), filtered, concentrated in vacuo, and purified by column chromatography (25% ethyl acetate/hexane) to give 64.3 mg (0.12 mmol, 78%) of (4-nitrophenyl)methylcarbamic acid 5-(*tert*-butyldiphenylsilanyloxy)-3-hydroxy-3-methylpentyl ester.

^{1}H NMR (300 MHz, CDCl$_3$): δ 8.20 (m, 2H), 7.39−7.70 (m, 12H), 4.41 (m, 2H), 3.80−4.1 (m, 2H), 3.39 (s, 3H), 1.60−2.0 (m, 4H), 1.28 (s, 3H), 1.10 (s, 9H). ^{13}C NMR (300 MHz, CDCl$_3$): δ 221, 142.5, 141.5, 136, 132.5, 129, 128, 124, 123, 72, 67, 64, 63, 42.5, 41, 38, 32, 27.5, 20, 16. HRMS (FAB): M + Cs$^+$ 683.1553 (expected), 683.1529 (observed).

To (4-nitrophenyl)methylcarbamic acid 5-(*tert*-butyldiphenylsilanyl-oxy)-3-hydroxy-3-methylpentyl ester (64.3 mg, 0.12 mmol) in dry ether (6 mL) containing 4.2 μL of water was added potassium *tert*-butoxide (103 mg, 0.84 mmol), and the mixture was stirred for 30 min. Workup with ether/saturated ammonium chloride furnished, after column chromatography (25% ethyl acetate/hexane), the known diol **15** (33.5 mg, 0.09 mmol, 75%). The conversion of diol **15** to mevalonolactone has been described.[26]

^{1}H NMR (300 MHz, CDCl$_3$): δ 7.36−7.80 (m, 10H), 3.80−4.05 (m, 4H), 1.88−2.10 (m, 2H), 1.65 (m, 2H), 1.06 (s, 12H).^{13}C NMR (300 MHz, CDCl$_3$): δ 158, 141, 137, 131, 128.5, 69, 64, 60, 44, 41, 33, 28, 24.5, 21. HRMS (FAB): M + Na$^+$ 395.2018 (expected), 395.2016 (observed).

Aldehyde **11** (179.5 mg, 0.58 mmol) in 4 mL of ether was added to a mixture of 1 M KMnO$_4$ (3.5 mL), phosphate buffer (pH 7.4, 2.3 mL), and *tert*-butyl alcohol (7 mL). The mixture was stirred for 5 min at room temperature. The mixture was treated with saturated aqueous Na_2SO_3, and the pH was adjusted to 3 (1 N HCl). Extraction with ether followed by drying with MgSO$_4$ and filtration gave, after column chromatography (1% acetic acid/ethyl acetate), 5-[(4-nitrophenyl)-methylcarbamoyloxy]-3-hydroxy-3-methylpentanoic acid (146 mg, 0.45 mmol, 77%).

^{1}H NMR (300 MHz, CDCl$_3$): δ 8.21 (m, 2H), 7.47 (m, 2H), 4.35 (t, J = 6 Hz, 2H), 3.38 (s, 3H), 2.54 (d, J = 9, 2H), 2.05 (m, 2H), 1.30 (s, 3H). ^{13}C NMR (300 MHz, CDCl$_3$): δ 220, 177, 143.5, 137, 125, 124, 75, 62, 50, 27, 24, 21. HRMS (FAB): M + Na$^+$ 349.1012 (expected), 349.1015 (observed).

5-[(4-Nitrophenyl)methylcarbamoyloxy]-3-hydroxy-3-methylpen-tanoic acid (20 mg, 0.064 mmol) in 2 mL of MeOH and 160 μL of NaOH (2 N) was stirred at room temperature overnight. The mixture was acidified to pH 4 with 2 M sulfuric acid at 0 °C and stirred for 3−4 h. Extraction with CH$_2$Cl$_2$ and column chromatography (50% ethyl acetate/hexane) furnished 1.4 mg (0.011 mmol, 17%) of mevalonolac-tone; the spectroscopic data are identical to those reported.[26]