α-Multistriatin: The First Total Synthesis of a Natural Product via Antibody Catalysis

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Abstract. The relevance of antibody catalysis to synthetic organic chemistry is demonstrated here by an efficient total synthesis of (–)-α-multistriatin, the aggregation pheromone of the European elm bark beetle, *Scolytus multistriatus*, which is the principal vector of the Dutch elm disease, responsible for the severe devastation of the elm population in Europe and North America. The key step in our synthesis of this natural product is an antibody-catalyzed, enantioselective protonolysis of an enol ether to produce a branched ketone. The latter is obtained with an (*S*) configuration in greater than 99% enantiomeric excess. Catalysis follows Michaelis–Menten kinetics (K_m = 230 μM, k_{cat} = 0.36 min^{-1}) and useful rate enhancement (k_{cat}/k_{un} = 65,000 at pH 6.5). This abzymic step is followed by twelve chemical steps, with all four asymmetric centers originating from the chirality achieved in the antibody-catalyzed reaction. That specific step is a unique example of a chemical transformation which is difficult to achieve either by an available synthetic methodology or via catalysis with a known enzyme. The synthetic pheromone has been checked in field experiments and found as active as the naturally occurring compound in attracting the European elm bark beetles.

INTRODUCTION

Since its inception not too long ago,[1] the science of catalytic antibodies has undergone a remarkable maturation process. From initial "proof of concept" and demonstration of fundamental, enzyme-like characteristics, catalytic antibodies have been shown to catalyze a broad scope of organic transformations, including difficult and unfavorable chemical reactions.[2] Moreover, very high levels of chemo-, regio-, and stereoselectivity have been achieved in most antibody-catalyzed reactions. Recent crystal structures show that these catalysts are highly homologous to natural enzymes but with the important advantage that they are induced in real time.[3] The relevance of the field to synthetic organic chemistry has been demonstrated recently by the ability to run these reactions with gram-scale quantities.[4] Here we go one step further and show for the first time that catalytic antibodies can be effectively used in natural product synthesis.[5] Enantioselective total synthesis of (–)-α-multistriatin, **1**, has been achieved with all four asymmetric centers originating from a key, antibody-catalyzed step.[6]

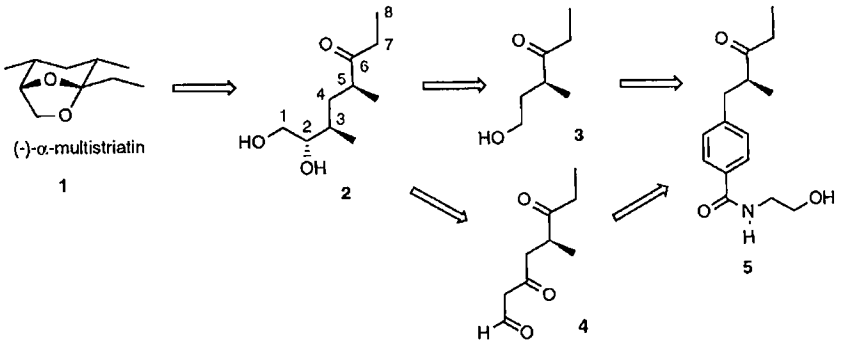
(–)-α-Multistriatin, is one of the three essential components of the aggregation pheromone of the European elm bark beetle, *Scolytus multistriatus* (Marsham), which is the principal vector of the fungus *Ceratocystis ulmi* which causes Dutch elm disease.[7] The severe devastation of the elm population in Europe and North America has resulted in extensive studies of the synthesis and field utilization of **1**.[8] Since the discovery of this pheromone by Silverstein and coworkers in 1975,[9] it has been the subject of numerous synthetic efforts.[10] Field experiments show that the inactive (+)-enantiomer of **1** inhibits the biological activity of the naturally-occurring (–)-enantiomer.[11] Thus, in order to achieve the required absolute configuration in all four asymmetric centers (1*S*, 2*R*, 4*S*, 5*R*), most of the previous syntheses of **1** employ enantiomerically pure natural products, such as D-glyceraldehyde,[10d] (*R*)-citronellol,[10e] D-glucose,[10j,k] D-galactose,[10m] and L-malic acid.[10o] Alternatively, the required optically active intermediates have been obtained either by resolution[10b,11] or by asymmetric epoxidation.[10n,p]

RESULTS AND DISCUSSION

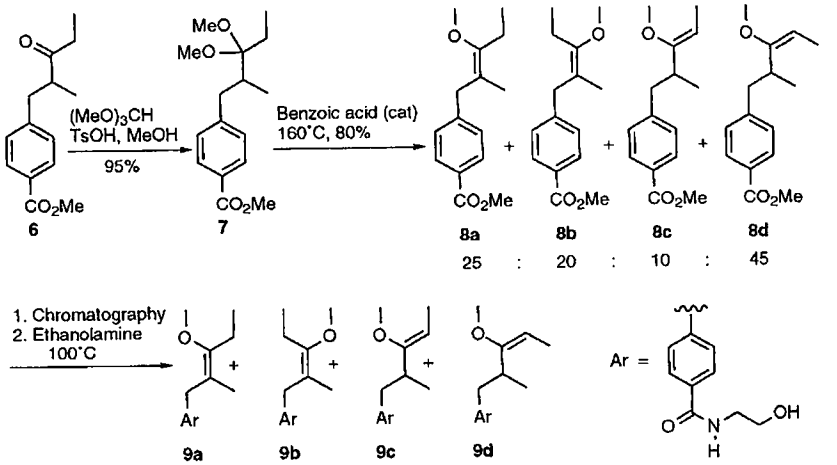
High enantiomeric purity of a tertiary carbon center adjacent to a carbonyl function is difficult to generate and hard to retain. Therefore, the molecular asymmetry in most reported syntheses of **1** has been derived from functionalized skeletons, such as **2**, where the two asymmetric centers at positions 2 and 3 are enantiomerically pure, while position 5 exists as a mixture of two epimers. This requires separation of the diastereomeric products by gas chromatography at the final step. In our retrosynthetic analysis (Scheme 1) we focused on the opportunity to obtain branched ketones such as **3** and **4** with high enantiomeric purity via antibody catalysis. Such ketones may be obtained from **5** via oxidative degradation of the aromatic ring. Based on previous results[12] with monoclonal antibody 14D9, we envisioned that ketone **5** could be obtained from antibody-catalyzed enantioselective protonolysis of the appropriate enol ether. Antibody 14D9 has already been proven an effective catalyst for hydrolysis of a variety of substrates that are structurally related to **5**, including a cyclic acetal,[13] ketals,[14] epoxides,[15] as well as enol ethers.[12,16]

All four isomeric substrates, **9a–d**, were conveniently prepared in a four-step sequence of very simple chemical transformations (Scheme 2). Thus, alkylation of 3-pentanone with methyl 4-bromomethylbenzoate afforded ketone **6,** which was then treated with trimethyl orthoformate and catalytic amounts of *p*-toluenesulfonic acid in methanol to give the corresponding ketal, **7**. The latter was heated in the presence of catalytic amounts of benzoic acid to produce, upon distillation (160 °C /1 mm), four isomeric enol ethers, **8a–d**, in a ratio of 25:20:10:45, respectively. These were heated with ethanolamine and purified by preparative HPLC to give compounds **9a–d**.

In acidic, aqueous media, all four isomers **9a–d** are hydrolyzed to racemic ketone **5**. Antibody 14D9 catalyzes this reaction under mildly acidic conditions (Scheme 3).[17] Interestingly, catalysis with the *Z* enol ether **9a** is much more effective (k_{cat}/k_{un} = 65,000) than with the *E* isomer, **9b**, (k_{cat}/k_{un} = 5,000).[18] This enzyme-like catalysis is evident from the observed Michaelis–Menten kinetics (**9a**: K_m = 230 mM, k_{cat} = 0.36 min^{-1} at pH 6.5; **9b**: K_m = 310 mM, k_{cat} = 0.044 min^{-1} at pH 6.0)



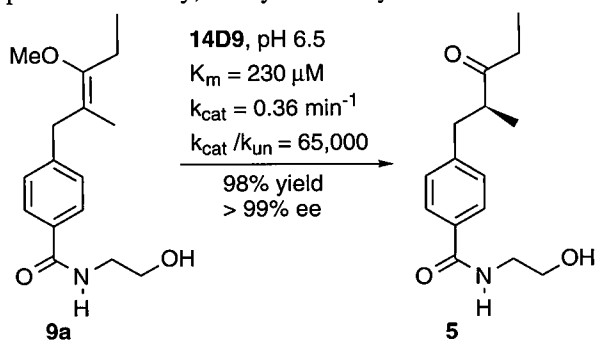
Scheme 1



Scheme 2

and from the fact that catalysis is totally inhibited in the presence of stoichiometric quantities (with respect to 14D9) of the methylpiperidinium hapten against which this antibody has been elicited.[13] Both **9a** and **9b** are hydrolyzed by 14D9 to produce ketone **5** with the same absolute configuration (*S*), probably due to the structural similarity between both substrates with respect to the preferred trajectory of proton approach to the homobenzylic carbon. Because the enantiomers of ketone **5** are not easily separable using chiral HPLC columns, we reduced them with sodium borohydride and obtained a diastereomeric mixture of alcohols **13** which are easily separated on a Chiracell OD-H HPLC column (Fig. 1). The absolute configuration was established by comparison with an enantiomerically enriched sample of (4*S*)-**13**. The latter was synthesized from the enantiomerically pure *N*-propionyl oxazolidones **10** (prepared from (1*S*,2*R*)-(+)-norephedrine, Scheme 4) using the Evans methodology, which is known to proceed with high diastereoselectivity and predictable absolute configuration.[19] As is seen in Fig. 1, ketone **5** is obtained from the antibody-catalyzed reaction with an (*S*) configuration in greater than 99% ee. This high enantioselectivity reflects the remarkably high rate acceleration in the case of **9a** (k_{cat}/k_{un} = 65,000) that allows the reaction to be driven to near completion with negligible interference of the background hydrolysis.

In addition to 14D9, several other monoclonal antibodies obtained from the same immunization[13] (13A8, 18E1, 13E7, 22H2, and 19C9) exhibited variable catalytic activity with **9a** but not with **9b–d**. Since rate enhancement with these antibodies was found to be lower than with antibody 14D9, the latter was selected for the large-scale synthesis. Because this specific reaction is not catalyzed by any known enzyme or other protein or any known biological component, there is no need to use a purified antibody.[20] Efficient catalysis is thus achieved with a partially purified antibody 14D9 which is precipitated from ascites fluid by saturated ammonium sulfate (SAS). Again, as is the case with the purified antibody, catalytic activity of this crude antibody is completely inhibited by the hapten, thereby ruling out any nonspecific catalysis by other components in the crude SAS fraction.



Scheme 3

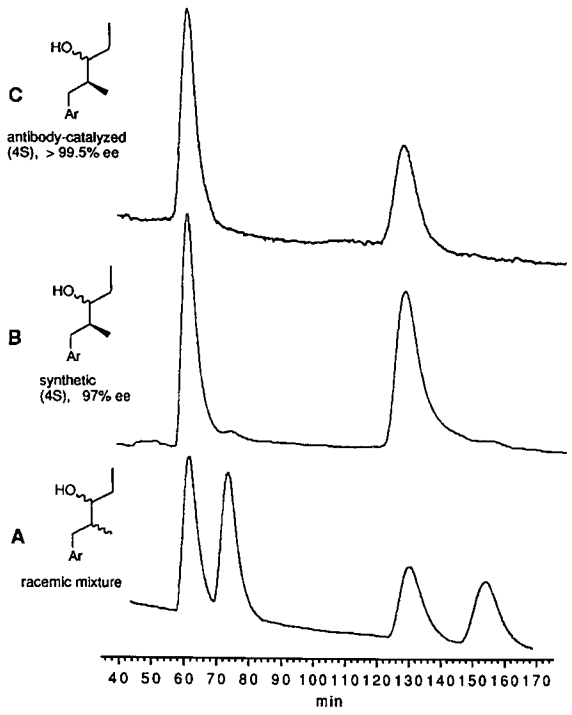
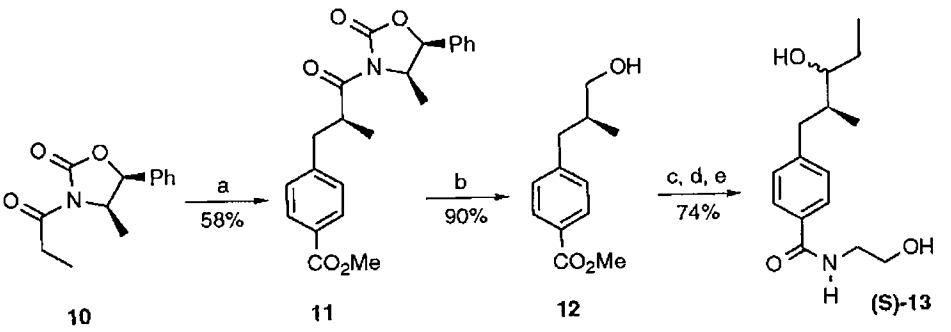


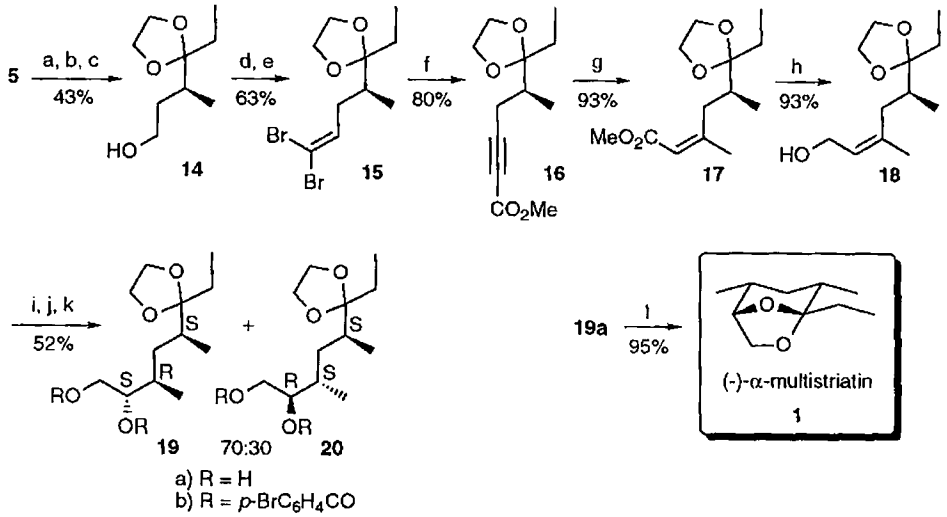
Fig. 1. Determination of absolute configuration and enantiomeric purity of ketone **5** produced in the antibody 14D9-catalyzed hydrolysis of **9a**: The ketone **5** was reduced with $NaBH_4$ to give the corresponding alcohol **13** as a mixture of epimers at the carbinol center. All analyses were carried out by HPLC (Perkin-Elmer 410 equipped with a UV detector, 254 nm) using a Chiracell OD-H column, Daicel Chemical Industries) with 5:95 isopropanol: hexane at a flow rate of 1 mL/min. (A) HPLC chromatogram of a racemic mixture of **13**. (B) Chromatogram of (4*S*)-**13** that was synthesized via the Evans method (19a) as shown in Scheme 4. (C) Chromatogram of **13** that was produced by 14D9-catalyzed hydrolysis of **9a** followed by borohydride reduction.

The Antibody-Catalyzed Step

We carried out the antibody-catalyzed reaction on a preparative scale using very simple organic-laboratory equipment.[4a] In each catalytic cycle a solution of the enol ether **9a** (180 mg, 0.65 mmol) in DMF (1 mL) was added to a solution of a crude SAS fraction of antibody 14D9 (22.5 mL containing 225 mg protein, 0.0015 mmol) in Bis-Tris buffer (50 mM, pH 6.5) and the mixture was stirred at 24 °C. Progress of the reaction was monitored by HPLC. It could also be seen visually, as the starting mixture was turbid-white (due to the lower solubility of the starting material than the product) and became clear as the reaction reached comple-



Scheme 4. Synthesis of enantiomerically enriched (*S*)-13. (a) LDA, THF, –78 °C, 30 min, methyl 4-(bromomethyl)ben-zoate, –20 °C, 5h. (b) $NaBH_4$, MeOH, 0 °C, 30 min. (c) PCC (2 equiv), CH_2Cl_2, Celite, 30 min. (d) EtMgBr (1 equiv), THF, –78 °C to room temp. (e) Ethanolamine, 100 °C, 1h.



Scheme 5. Stereoselective synthesis of α-multistriatin. Key: (a) $RuCl_3$, $NaIO_4$, CCl_4, CH_3CN, H_2O. (b) Ethylene glycol, PPTS, benzene. (c) $LiAlH_4$, ether. (d) PCC, CH_2Cl_2. (e) CBr_4, PPh_3, CH_2Cl_2. (f) *n*-BuLi, $NCCO_2Me$, THF. (g) MeCu, TMEDA, ether. (h) DIBAL-H, THF, toluene. (i) BH_3-SMe_2, then H_2O_2/NaOH. (j) *p*-BrC_6H_4COCl, Et_3N, DMAP, CH_2Cl_2, column chromatography. (k) $LiAlH_4$, ether.(l) PPTS, CH_2Cl_2.

tion. The reaction was interrupted after 60 h at 80% conversion by transferring the mixture into a cellulose dialysis bag, which allows diffusion of molecules smaller than 12–14 kDa, and dialyzed into 500 mL of the same buffer over 16 h, a technique that was proven useful both with enzymes[21] and antibodies.[4a] The antibody solution was taken to the next catalytic cycle with a fresh solution of **9a**. As reported in the previous large-scale use of 14D9, only minor deterioration of catalytic activity could be observed over the first five cycles of the reaction.[4a] Thus, in five reaction cycles 900 mg (3.25 mmol) substrate was treated with 0.0015 mmol of crude antibody to give recovered **9a** (175 mg) and pure ketone **5** (591 mg, 86% based on recovered starting material). Enantiomeric purity of this product (95% ee) was found to be somewhat lower than the 99% ee observed in the small-scale preparation.

The Chemical Steps

An "atom economy" approach[22] could take advantage of the fact that all carbon atoms needed to construct the final skeleton of **1** already exist in compound **5**. Accordingly, we were able to achieve partial degradation of the aromatic ring within **5** by ketalization of the ketone, followed by $LiAlH_4$-reduction of the amide to amine, Birch reduction of the aromatic ring to a substituted cyclohexa-1,4-diene, and ozonolysis to give the ketal derivative of **4**. This approach, however, is too long and inefficient, as conversion of **4** to the desired skeleton **2**, in particular, requires many additional steps with difficult control of stereochemistry. Therefore, this strategy was abandoned in favor of the one based on complete degradation of the aromatic ring, followed by gradual addition of the missing carbon atoms (Scheme 5).

Thus, ketone **5** was treated with $RuCl_3$ and sodium

periodate in a biphasic system (carbon tetrachloride and acetonitrile–water) at room temperature.[23] The resultant keto-acid was then converted to a ketal-ester by reaction with ethylene glycol and catalytic amounts of pyridinium *p*-toluenesulfonate (PPTS) in benzene. Reduction of the resultant carboxylic ester with $LiAlH_4$ in ether afforded alcohol **14**, whose enantiomeric purity was determined by ^1H NMR of the corresponding (*R*)-Mosher ester.[24] Thus, esterification of **14** was carried out with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride and dimethylaminopyridine in dichloromethane. The integration ratio between the two doublets at 0.93 (representing the *S* enantiomer) and at 0.92 (representing the *R* enantiomer) was found to be 96:4, i.e., 92% ee. This reflects only minor loss of enantiomeric purity throughout the three-step degradation procedure. Alcohol **14** was oxidized with pyridinium chlorochromate in dichloromethane to the corresponding aldehyde. The latter was treated with a solution of triphenylphosphine and carbon tetrabromide in dichloromethane to give the dibromoalkene, **15**.[25] Treatment of **15** with *n*-butyllithium and methyl cyanoformate in hexane–THF produced the substituted methyl propargylate **16**.[25] Reaction of the latter with "MeCu" (prepared from methyllithium and CuI in THF and tetramethylethylenediamine) afforded geometrically pure (*Z*) α,β-unsaturated ester **17**.[26] Reduction of this ester with diisobutylaluminum hydride in toluene–THF afforded the corresponding allylic alcohol, **18**.

Hydroboration of allylic alcohols is known to be an efficient, highly regioselective transformation due to initial complexation of boron to the alcohol.[27] Thus, treatment of **18** with a solution of borane–dimethyl sulfide complex in THF, followed by oxidation with basic (NaOH, 3N) hydrogen peroxide produced a 70:30 mixture of two diastereomeric products: **19a** (having the required 2*S*,3*R*,5*S* configuration) and its 2*R*,3*S*,5*S*-diastereomer, **20a**, respectively. Esterification with 4-bromobenzoyl chloride produced the corresponding bis-bromobenzoate derivatives, **19b** and **20b**, which were easily separated by column chromatography. The purified diol **19a** was then recovered via reductive cleavage with $LiAlH_4$. Finally, treatment of **19a** with catalytic amounts of pyridinium *p*-toluenesulfonate in dichloromethane followed by kugelrohr distillation at 110 °C afforded (–)-α-multistriatin, **1,** in the form of a colorless oil.

The synthetic pheromone **1** has been checked in field experiments and found as active as the naturally occurring compound in attracting the European elm bark beetles into traps loaded with a mixture of **1** with (–)-α-cubebene (a host-produced component) and (–)-4-methylheptan-3-ol.

CONCLUSION

The relevance of antibody catalysis to synthetic organic chemistry has been demonstrated here by an efficient total synthesis of an important, biologically active, natural product. All four asymmetric centers originate from a key, antibody-catalyzed protonolysis of an enol ether. That specific step is a unique example of a chemical transformation which is difficult to achieve either by an available synthetic methodology or via catalysis with a known enzyme.[28]

The synthesis of natural products remains the ultimate testing ground for new concepts in organic chemistry. This has been the case, for example, with the advent of organometallic chemistry throughout the past four decades. Thus, the key point in the present study is not simply that one can make α-multistriatin, or even that this is now the best way to synthesize the compound, but rather that catalytic antibodies perform competitively in the important testing ground of natural product synthesis.

EXPERIMENTAL SECTION

General Methods

^1H and ^{13}C NMR spectra were measured in $CDCl_3$ at 400 and 100 MHz, respectively. Positive ion mass spectra, using the fast ion bombardment (FIB) technique, were obtained on a VG ZAB-VSE double focusing, high resolution mass spectrometer equipped with either a cesium or sodium ion gun. Infrared spectra were measured with a Perkin-Elmer 1600 (FTIR) spectrometer. Optical rotations were measured in a one decimeter (1 mL) cell using Autopol III automatic polarimeter. TLC was performed on glass sheets precoated with silica gel (Merck, Kieselgel 60, F254, Art. 5715). Column chromatographic separations were performed on silica gel (Merck, Kieselgel 60, 70–230 mesh, Art. 9385) at atmospheric pressure. THF was dried by distillation over sodium benzophenone ketyl. (*R*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (Mosher's acid) was purchased from Aldrich.

1-(4'-Methoxycarbonylphenyl)-2-methylpentan-3-one, 6

A solution of *n*-BuLi (2.5 M in hexane, 66 mL, 0.165 mol) was slowly added to a solution of diisopropylamine (23.6 mL, 0.18 mol) in dry THF (150 mL) at 0 °C. The mixture was cooled to –78 °C, a solution of 3-pentanone (12.92 g, 0.15 mol) in dry THF (25 mL) was added dropwise, and the mixture was stirred at the same temperature for 2.5 h. A solution of methyl 4-bromomethyl benzoate (34.36 g, 0.15 mol) and HMPA (28.7 mL, 0.165 mol) in dry THF (35 mL) was added, the mixture was stirred at –78 °C for 3 h, then at room temperature for 16 h, and then worked up with ether and water. The residue was distilled (200–220 °C/20 mm) to give pure **6** (23.77 g). ^1H NMR: 7.95 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 3.91 (s, 3H), 3.03 (dd, J = 13.4, 7.4 Hz, 1H), 2.86 (m, 1H), 2.62 (dd, J = 13.4, 7.2 Hz, 1H), 2.45 (dq, J = 17.9, 7.2 Hz, 1H), 2.24 (dq, J = 17.9, 7.2 Hz 1H), 1.10 (d, J =

7.2 Hz, 3H), 0.97 (t, J = 7.2 Hz, 3H).

1-(4'-Methoxycarbonylphenyl)-2-methyl-3,3-dimethoxypentane, 7

A mixture of **6** (23.75 g, 0.101 mol), *p*-toluenesulfonic acid (1 g), trimethylorthoformate and methanol (2:1, 100 mL) was stirred at 60 °C for 2 h, then worked up by removal of solvents under reduced pressure, addition of triethylamine followed by saturated aqueous sodium bicarbonate, and extraction with ether to produce the corresponding crude dimethyl ketal, **7** (29 g) which was taken to the next step without further purification. ^{1}H NMR: 7.94 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 3.88 (s, 3H), 3.26 (s, 3H), 3.19 (s, 3H), 3.14 (dd, J = 13.0, 2.3 Hz, 1H), 2.28 (dd, J = 13.4,11.5 Hz, 1H), 2.12 (m, 1H). 0.97 (t, J = 7.7 Hz, 3H), 0.83 (d, J = 6.9 Hz, 3H).

(Z) and (E) 1-[4'-(2-Hydroxyethylamino) carbonylphenyl] -2-methyl-3-methoxypent-2(or 3)-ene, 9a–9d

Benzoic acid (1 g) was added to the crude ketal **7** (29 g, 100 mmol) and the mixture was distilled (160 °C/1mm) to give a mixture of all four isomeric enol ethers **8a–d** in the ratio of 25:20:45:10, respectively, as well as some ketone **6** (together, 25 g). This mixture can be separated by column chromatography into two major fractions, one comprising enol ethers **8a** and **8b** and the other containing enol ethers **8c**, **8d**, and ketone **6**. The mixture of enol ethers **8a–d** was heated with ethanolamine (10 mL) at 100 °C for 1 h and then worked up with water and dichloromethane. Purification by preparative HPLC afforded pure samples of all four isomeric substrates **9a–d**.

^{1}H NMR of **9a**: 7.66 (d, J = 8.2 Hz, 2H), 7.20 (d, J = 8.2 Hz, 2H), 6.79 (m, 1H), 3.77 (m, 2H), 3.57 (m, 2H), 3.49 (s, 3H), 3.43 (s, 2H), 3.23 (br s, 1H), 2.24 (q, J = 7.5 Hz, 2H), 1.48 (s, 3H), 1.04 (t, J = 7.5 Hz, 3H). ^{13}C NMR: 168.72, 152.34, 145.59, 131.42, 128.77, 126.92, 114.16, 62.29, 56.48, 42.79, 36.67, 19.70, 16.04, 12.20 ppm. IR (neat): 3333.3, 2933.3, 1635.9, 1543.6, 1502.6. HRMS: calcd for $C_{16}H_{24}O_3N$ ($M+H^+$), 278.1756, found 278.1745.

^{1}H NMR of **9b**: 7.70 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 6.79 (m, 1H), 3.83 (m, 2H), 3.62 (m, 2H), 3.53 (s, 3H), 3.35 (s, 2H), 2.90 (t, J = 2.1 Hz, 1H), 2.31 (q, J = 7.5 Hz, 2H), 1.58 (s, 3H), 1.07 (t, J = 7.5 Hz, 3H).

^{1}H NMR of **9c**: 7.69 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 8.0 Hz, 2H), 6.57 (m, 1H), 4.60 (q, J = 6.8 Hz, 1H), 3.85 (m, 2H), 3.64 (m, 2H), 3.62 (s, 3H), 2.92 (m, 1H), 2.55 (m, 3H), 1.58 (d, J = 6.8 Hz, 3H), 0.97 (d, J = 6.4 Hz, 3H).

^{1}H NMR of **9d**: 7.64 (d, J = 8.0 Hz, 2H), 7.13 (d, J = 8.0 Hz, 2H), 6.88 (m, 1H), 4.22 (q, J = 6.8 Hz, 1H), 3.75 (m, 2H), 3.56 (m, 2H), 3.46 (br s, 1H), 3.41 (s, 3H), 2.87 (m, 1H), 2.77 (dd, J = 12.9, 9.0 Hz, 1H), 2.60 (dd, J = 12.9, 8.0 Hz, 1H), 1.30 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H).

(S) 1-[4'-(2-Hydroxyethylamino) carbonylphenyl] -2-methylpentan-3-one, 5

A solution of the **9a** (180 mg, 0.65 mmol) in DMF (1 mL) was added to a solution of antibody 14D9 (225 mg, 0.0015 mmol) in 22.5 mL Bis-Tris buffer (50 mmol, pH 6.5) and the mixture was stirred at 24 °C. Progress of the reaction was followed by HPLC.[17] When the reaction reached 80% completion (after 60 h) the mixture was placed in a dialysis bag and dialyzed against 500 mL of the same buffer solution for 16 h. The product was extracted with dichloromethane from the buffer solution after being saturated with NaCl. The antibody solution was transferred from the dialysis bag and mixed with a fresh solution of **9a**. This procedure has been repeated five times with **9a** (total 900 mg, 3.25 mmol), producing a mixture of **5** and **9a**. Separation by column chromatography (silica gel, hexane:ethyl acetate 1:1) afforded recovered **9a** (175 mg, 19%) and pure ketone **5** (591 mg, 69%, 87% on the basis of consumed starting material). $[\alpha]_D$: +46.6° (c = 1.38, $CHCl_3$). ^{1}H NMR: 7.69 (d, J = 8.2 Hz, 2H), 7.17 (d, 8.2 Hz, 2H), 6.96 (t, J = 4.8 Hz, 1H), 3.79 (t, J = 4.8 Hz, 2H), 3.58 (q, J = 4.8 Hz, 2H), 3.00 (dd, J = 13.4, 7.5 Hz, 1H), 2.85 (m, 1H), 2.60 (dd, J = 13.4, 7.0 Hz, 1H), 2.45 (dq J = 17.9, 7.3 Hz, 1H), 2.25 (dq J = 17.9, 7.3 Hz, 1H), 1.08 (d, J = 6.8 Hz, 3H), 0.96 (t, J = 7.2 Hz, 3H). ^{13}C NMR: 214.46, 168.20, 143.45, 131.81, 128.71, 126.90, 61.28, 47.21, 42.49, 38.49, 34.66, 16.37, 7.28 ppm. HRMS: calcd for $C_{15}H_{22}O_3N$ ($M+H^+$), 264.1600, found 264.1605.

(S) 2-Ethyl-2-(1'-hydroxybut-3'-yl)-1,3-dioxolane, 14

A mixture of **5** (591 mg, 2.25 mmol), $RuCl_3$ (50 mg, 0.24 mmol), sodium periodate (10.16 g, 47.5 mmol) in CCl_4 (15 mL), acetonitrile (15 mL) and water (22.5 mL) was stirred at 24 °C for 40 h. The mixture was extracted with ethyl acetate, the organic phase was passed through a short bed of Celite, concentrated, and passed through a short bed of silica gel to give 230 mg of crude carboxylic acid. The latter was mixed with ethylene glycol (1 mL) and PPTS (50 mg) in benzene (20 mL) and refluxed for 40 h with azeotropic distillation of water. The mixture was worked up with saturated aqueous sodium bicarbonate and ether, and solvents were removed under reduced pressure. The residue was dissolved in dry ether (5 mL), cooled to 0 °C, and $LiAlH_4$ (120 mg, 3 mmol) was slowly added. The mixture was stirred at 24 °C for 2 h, then quenched with the slow addition of water, dried over Na_2SO_4, concentrated, and the residue was purified over silica gel to give alcohol **14** (170 mg, 43%). $[\alpha]_D$: –11.6 (c = 1.58, $CHCl_3$); ^{1}H NMR: 3.95 (br s, 4H), 3.72 (m, 1H), 3.60 (m, 1H), 2.26 (br s, 1H), 1.94 (m, 1H), 1.79 (m, 1H), 1.66 (q, J = 7.4 Hz, 2H), 1.42 (m, 1H). 0.95 (d, J = 7.4 Hz, 3H), 0.87 (d, J = 7.4 Hz, 3H); ^{13}C NMR: 113.92, 65.27, 65.22, 61.37, 36.69, 34.58, 26.33, 15.05, 7.41 ppm.

(S) 2-Ethyl-2-(1',1'-dibromopent-1'-en-4'-yl)-1,3-dioxolane, 15

Alcohol **14** (170 mg, 0.98 mmol) was dissolved in dichloromethane (10 mL) and PCC (430 mg, 2 mmol), the mixture was stirred for 1.5 h, filtered through a short bed of silica gel, and the solvent was removed to give the corresponding aldehyde (165 mg). The latter was redissolved in dichloromethane (5 mL), the solution was cooled to 0 °C, triphenylphosphine (2.1g, 8 mmol) and CBr_4 (1.33 g, 4 mmol) were added, the mixture was stirred for 10 min, and then filtered through silica gel (that was pretreated with triethylamine) using dichloromethane. Solvent was removed under reduced pressure to produce **15** (200 mg, 63%). $[\alpha]_D$:–8.5 (c = 2.14, $CHCl_3$). ^{1}H NMR: 6.43 (t, J = 6.6 Hz, 1H), 3.95 (m, 4H), 2.32 (m, 1H), 1.97 (m, 2H), 1.64 (m, 2H), 0.96 (d, J = 6.6 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H). ^{13}C NMR: 138.14, 113.28, 88.69, 65.34, 65.31, 38.61, 34.90, 26.96, 14.41, 7.55 ppm. IR: 2971.0,

2881.0, 1463.2, 1057.1, 923.5, 781.9 cm^{-1}. HRMS: calcd for $C_{10}H_{17}O_2Br_2$ ($M+H^+$), 326.9595, found 326.9607.

*(S) 2-Ethyl-2-(1'-methoxycarbonylpent-2'-yn-5'-yl)-1,3-dioxolane, **16***

n-BuLi (0.5 mL, 2.5 M in hexane, 1.25 mmol) was added to a solution of **15** (200 mg, 0.61 mmol) in THF (2 mL), the mixture was stirred at –78 °C for 1 h, then at 24 °C for 1 h, and then cooled to –78 °C. Methyl cyanoformate (0.085 mL, 1 mmol) was added, the mixture was stirred for 0.5 h and then worked up with ether and water and the residue was filtered through silica gel to give pure alkyne **16** (110 mg, 80%). $[\alpha]_D$: –22.3 (c = 1.44, $CHCl_3$). ^1H NMR: 3.92 (m, 4H), 3.73 (s, 3H), 2.58 (dd, *J* = 16.3, 2.8 Hz, 1H), 2.12 (m, 2H), 1.60 (m, 2H), 1.07 (d, *J* = 6.8 Hz, 3H), 0.86 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR: 176.46, 154.17, 112.61, 89.46, 65.33, 65.29, 52.46, 38.43, 26.83, 20.66, 14.40, 7.48 ppm. IR: 2971.3, 2883.3, 2237.4, 1717.8, 1457.6, 1436.7 cm^{-1}. HRMS: calcd for $C_{12}H_{19}O_4$ ($M+H^+$), 227.1283, found 227.1283.

*(S) (Z)-2-Ethyl-2-(1'-methoxycarbonyl-2'-methylpent-1'-en-4'-yl)-1,3-dioxolane, **17***

A solution of methyllithium (1.4 M in ether, 0.5 mL, 0.7 mmol) was added dropwise to a heterogeneous mixture of CuI (133 mg, 0.7 mmol) in THF (2 mL) at –40 °C. TMEDA (0.13 mL, 0.84 mmol) was added and the mixture was stirred for 45 min. A solution of **16** (110 mg, 0.49 mmol) was added dropwise, the mixture was stirred for 30 min, heated to –30 °C and then worked up with saturated aqueous NH_4Cl and ether, washed with water and filtered through silica gel to give pure alkene **17** (110 mg, 93%). $[\alpha]_D$: +27.4 (c = 2.10, $CHCl_3$). ^1H NMR: 5.72 (t, *J* = 1.2 Hz, 1H), 3.94 (m, 4H), 3.65 (s, 3H), 3.11 (dd, *J* = 12.4, 11.0 Hz, 1H), 2.37 (dd, *J* = 12.4, 3.9 Hz, 1H), 2.03 (m, 1H), 1.85 (d, *J* = 1.2 Hz, 3H), 1.69 (q, *J* = 7.4 Hz, 2H), 0.89 (t, *J* = 7.4 Hz, 3H), 0.85 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR: 166.75, 159.49, 117.11, 113.72, 65.40, 65.36, 50.71, 38.18, 34.04, 26.59, 24.99, 13.62, 7.37 ppm. IR: 2974.7, 2880.3, 1718.7, 1646.9, 1458.3, 1437.2 cm^{-1}. HRMS: calcd for $C_{13}H_{23}O_4$ ($M+H^+$), 243.1596, found 243.1594.

*(S) (Z)-2-Ethyl-2-(1'-hydroxy-3'-methylhex-2'-en-5'-yl)-1,3-dioxolane, **18***

DIBAL-H (1 M in toluene, 1.5 mL, 1.5 mmol) was added to a solution of **17** (110 mg, 0.45 mmol) in THF (2 mL) at –30 °C and the mixture was stirred at the same temperature for 1 h. Saturated aqueous NH_4Cl (2 mL) and ether (10 mL) were added, the mixture was stirred for 1h and then filtered through a short bed of silica gel, affording pure alcohol **18** (90 mg, 93%). $[\alpha]_D$: +6.5 (c = 1.50, $CHCl_3$). ^1H NMR: 5.48 (tt, *J* = 7.1, 1.2 Hz, 1H), 4.17 (dd, *J* = 12.2, 7.6 Hz, 1H), 4.06 (dd, *J* = 12.2, 6.6 Hz, 1H), 3.96 (m, 4H), 2.16 (dd, *J* = 12.9, 3.3 Hz, 1H), 2.03 (dd, *J* = 12.9, 10.6 Hz, 1H), 1.91 (m, 1H), 1.71 (d, *J* = 1.2 Hz, 3H), 1.65 (qd, *J* = 7.5, 2.9 Hz, 2H), 1.53 (br s, 1H), 0.88 (t, *J* = 7.4 Hz, 3H), 0.83 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR: 138.74, 125.55, 113.85, 65.40, 65.32, 59.02, 37.61, 33.21, 26.88, 23.47, 13.92, 7.53 ppm; IR: 3409.1, 2968.2, 2880.6, 1665.9, 1462.8 cm^{-1}; HRMS: calcd for $C_{12}H_{22}O_3Na$ ($M+Na^+$), 237.1467, found 237.1470.

*(2S,4R,5S) and (2S,4S,5R) 2-Ethyl-2-(1',2'-dihydroxy-3'-methylhex-5'-yl)-1,3-dioxolane, **19a and 20a***

A solution of borane–dimethylsulfide complex (2 M in THF, 0.25 mL) was added to a solution of **18** (90 mg, 0.42 mmol) in THF (1 mL) at 0 °C and the mixture was stirred overnight at the same temperature. Aqueous NaOH (3N, 0.25 mL) was added, followed by dropwise addition of H_2O_2 (30%, 0.25 mL). The mixture was stirred at 60 °C for 1h, then worked up with ether and water, and the ether extract was purified by filtration through silica gel (hexane:ethyl acetate 1:1) to produce a mixture of two diols, **19a** and **20a** (60 mg, 62%) in a ratio of 7:3, respectively. Treatment of the mixture (60 mg, 0.26 mmol) with 4-bromobenzoyl chloride (283 mg, 1.3 mmol), triethylamine (2 mL), and DMAP (40 mg) in dichloromethane (2 mL) afforded the corresponding mixture of the corresponding bis-bromobenzoate derivatives. **19b**: ^1H NMR: 7.87 (d, *J* = 8.7 Hz, 2H), 7.79 (d, *J* = 8.7 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 2H), 5.51 (m, 1H), 4.50 (m, 2H), 3.83 (m, 2H), 3.79 (m, 2H), 2.07 (m, 1H), 1.89 (m, 1H), 1.72 (m, 1H), 1.57 (m, 2H), 1.11 (d, *J* = 6.8 Hz, 3H), 1.01 (m, 1H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.78 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR: 165.51, 165.25, 131.73, 131.70, 131.06, 131.02, 128.99, 128.58, 128.21, 128.13, 113.71, 73.15, 65.19, 65.14, 65.11, 36.73, 35.20, 32.32, 25.69, 16.09, 15.12, 7.05 ppm. **20b**: ^1H NMR: 7.87 (d, *J* = 8.7 Hz, 2H), 7.80 (d, *J* = 8.7 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.52 (d, *J* = 8.7 Hz, 2H), 5.40 (m, 1H), 4.59 (dd, *J* = 11.9, 3.2 Hz, 1H), 4.47 (dd, *J* = 11.9, 7.7 Hz, 1H), 3.91 (m, 4H), 2.05 (m, 1H), 1.85 (m, 1H), 1.61 (m, 2H), 1.46 (m, 1H), 1.31 (m, 1H), 1.03 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.85 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR: 165.54, 165.33, 131.76, 131.72, 131.11, 128.94, 128.60, 128.24, 128.21, 113.78, 76.00, 65.39, 65.35, 64.59, 36.50, 34.01, 32.28, 26.56, 14.49, 14.00 7.41 ppm.

Compounds **19b** (105 mg) and **20b** (40 mg) were separated by column chromatography (silica gel hexane:ethyl acetate 9:1). $LiAlH_4$ (20 mg) was added to a solution of **19b** (105 mg) in ether (2 mL) at 0 °C, the mixture was stirred at 24 °C for 1 h, and then worked up with water and ether, dried over sodium sulfate and filtered through silica gel to give pure diol **19a** (38 mg). ^1H NMR of **19a**: 3.95 (m, 4H), 3.62 (m, 3H), 2.62 (br s, 1H), 2.33 (br s, 1H), 1.83 (m, 1H), 1.65 (m, 5H), 0.93 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 6.7 Hz, 3H), 0.87 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR: 114.43, 73.46, 65.39, 65.33, 65.19, 36.82, 35.33, 33.70, 26.21, 15.82, 15.43, 7.39 ppm. HRMS: calcd for $C_{12}H_{24}O_4Na$ ($M+Na^+$), 255.1572, found 255.1569.

*α-Multistriatin, **1***

A solution of **19a** (38 mg, 0.16 mmol) and PPTS (5 mg) in dichloromethane (1 mL) was stirred at 24 °C overnight. The resultant product was passed through a short bed of silica gel using dichloromethane, solvents were removed under reduced pressure, and the residue was kugelrohr-distilled (100–120 °C/ 30 mm, bath temperature) to give pure **1** (20 mg). The synthetic pheromone **1** was found to be identical (NMR, IR, MS, $[\alpha]_D$) to the naturally occurring compound.[9k] It has been checked in field experiments by Dr. Stephen A. Teale of the State University of New York at Syracuse, and was found to

be as active as the naturally occurring compound in attracting the European elm bark beetles into traps loaded with a mixture of **1** with (–)-α-cubebene (a host-produced component) and (–)-4-methylheptan-3-ol.

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REFERENCES AND NOTES

(1) (a) Tramontano, A.; Janda, K.; Lerner, R.A. *Science* **1986**, *234*, 1566–1570. (b) Pollack, S.J.; Jacobs, J.W.; Schultz, P.G. *Science* **1986**, *234*, 1570–1573.

(2) (a) Lerner, R.A.; Benkovic, S.J.; Schultz, P.G. *Science* **1991**, *252*, 659. (b) Schultz, P.G.; Lerner, R.A. *Acc. Chem. Res.* **1993**, *26*, 391. (c) Schultz, P.G.; Lerner, R.A. *Science* **1995**, *269*, 1835.

(3) (a) Haynes, M.R.; Stura, E.A.; Hilvert D.; Wilson, I.A. *Science* **1994**, *263*, 646. (b) Zhou, G.W.; Guo, J.; Fetterick, R.J.; Scanlon, T.S. *Science* **1994**, *265*, 1059. (c) Golinelli-Pimpaneau, B.; Gigant, B.; Bizebard, T.; Navaza, J.; Saludjian, P.; Zemel, R.; Tawfik, D.S.; Eshhar, Z.; Green, B.S.; Knossow, M. *Structure* **1994**, *2*, 175.

(4) (a) Reymond, J.-L.; Reber, J.-L.; Lerner, R.A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 475. (b) Shevlin, C.G.; Hilton, S.; Janda, K.D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 297.

(5) For a preliminary report of this work, see: Sinha, S.C., Keinan, E. *J. Am. Chem. Soc.* **1995**, *117*, 3653.

(6) As all reagents used in the synthesis are achiral, the only source of asymmetry in the molecule is the antibody-catalyzed reaction. The other three asymmetric centers are produced later by chirality transfer where only the relative stereochemistry is controlled.

(7) Lanier, G.N.; Silverstein, R.M.; Peacock, J.W. In *Perspectives of Forest Entomology*; Anderson, J.F.; Kaya, H.K., Eds:, Academic Press: New York, 1976; Chapter 12.

(8) Lanier, G.N.; Gore, W.E.; Pearce, G.T.; Peacock, J.W.; Silverstein, R.M. *J. Chem. Ecol.* **1977**, *3*, 1.

(9) Pearce, G.T.; Gore, W.E.; Silverstein, R.M.; Peacock, J.W.; Cuthbert, P.A.; Lanier, G.N.; Simeone, J.B. *J. Chem. Ecol.* **1975**, *1*, 115.

(10) (a) Gore, W.E.; Pearce, G.T.; Silverstein, R.M. *J. Org. Chem.* **1975**, *40*, 1705. (b) Pearce, G.T.; Gore, W.E.; Silverstein, R.M. *J. Org. Chem.* **1976**, *41*, 2797. (c) Elliot, W.J.; Fried, J. *J. Org. Chem.* **1976**, *41*, 2475. (d) Mori, K. *Tetrahedron* **1976**, *32*, 1979. (e) Cernigliaro, G.J.; Kocienski, P.J. *J. Org. Chem.* **1977**, *42*, 3622. (f) Sum, P.-E.; Weiler, L. *Can. J. Chem.* **1978**, *56*, 2700. (g) Bartlett, P.A.; Myerson, J. *J. Org. Chem.* **1979**, *44*, 1625. (h) Fitzsimmons, B.J.; Plaumann, D.E.; Fraser-Reid, B. *Tetrahedron Lett.* **1979**, 3925. (i) Marino, J.P.; Abe, H. *J. Org. Chem.* **1981**, *46*, 5379. (j) Sum, P.-E.; Weiler, L. *Can. J. Chem.* **1982**, 60, *327*. (k) Plaumann, D.E.; Fitzsimmons, B.J.; Ritchie, B.M.; Fraser-Reid, B. *J. Org. Chem.* **1982**, *47*, 941. (l) Walba, D.M.; Wand, M.D. *Tetrahedron Lett.* **1982**, 23, 4995. (m) Lagrange, A.; Olesker, A.; Soares Costa, S.; Lukacs, G.; Thang, T.T. *Carbohydrate Res.* **1982**, *110*, 159. (n) Helbig, W. *Liebigs Ann. Chem.* **1984**, 1165. (o) Larcheveque, M.; Henrot, S. *Tetrahedron* **1987**, *43*, 2303. (p) Mori, K.; Seu, Y.-B. *Tetrahedron* **1988**, *44*, 1035.

(11) Elliott, W.J.; Hromnak, G.; Fried, J.; Lanier, G.N. *J. Chem. Ecol.* **1979**, *5*, 279.

(12) (a) Reymond, J.-L.; Lerner, R.A. *J. Am. Chem. Soc.* **1992**, *114*, 2257. (b) Reymond, J.-L.; Jahangiri, G.K.; Stoudt, C., Lerner, R.A. *J. Am. Chem. Soc.* **1993**, *115*, 3909.

(13) Reymond, J.-L.; Janda, K.D.; Lerner, R.A. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1711.

(14) Sinha, S.C.; Keinan, E.; Reymond, J.-L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11910.

(15) Sinha, S.C.; Keinan, E.; Reymond. J.-L. *J. Am. Chem. Soc.* **1993**, *115*, 4893.

(16) Shabat, D.; Itzhaky, H.; Reymond, J.-L.; Keinan, E. *Nature*, **1995**, *374*, 143.

(17) Assay conditions: antibody (0.5 μM), substrate (50–500 μM), 1,3-bis[tris(hydroxymethyl) methylamino]propane-buffered saline (50 mM, pH 6.5, 50 mM NaCl). All reactions were carried out at 24 °C and monitored at 254 nm by RP-HPLC (Hitachi L-6200A equipped with a Spherisorb column (25 cm × 4.6 mm, C18, 5 μm) using 42:58 acetonitrile:water at 0.8 mL/min.

(18) In one experiment, a mixture of all four compounds, **9a–d**, was subjected to antibody-catalyzed hydrolysis, resulting in complete, selective consumption of **9a** with essentially no change in the concentration of **9b** and small decrease of **9c,d**. However, since enol ethers **9c,d** are hydrolyzed approximately ten times faster than **9a,b** under the same buffer conditions, in order to achieve high ee and avoid contamination of the product by racemic **5**, it was necessary to remove **9b–d** from the mixture before the antibody-catalyzed step.

(19) (a) Evans, D.A.; Ennis, M.D.; Mathre, D.J. *J. Am. Chem. Soc.* **1982**, *104*, 1737. (b) Evans, D.A.; Bartoli, J. *Tetrahedron Lett.* **1982**, *23*, 807. (c) Correa, A.; Denis, J.-N; Greene, A.E. *Synth. Commun.* **1991**, *21*, 1. (d) Shin, I.; Zhou, H.-Q.; Que, N.L.S.; Liu, H.-W. Swedenborg, P.D.; Jones, R.L. *J. Org. Chem.* **1993**, *58*, 2923. The absolute configuration at the homobenzylic position was assigned on the basis of the many examples reported by Evans, including a closely related asymmetric alkylation reaction with unsubstituted benzyl bromide.

(20) Antibody 14D9 is the product of a murine hybridoma cell line. It is made by producing ascites fluid from this cell line followed by ammonium sulfate precipitation, anion exchange, and affinity chromatography with immobilized G protein: (a) Köhler, G.; Milstein, C. *Nature*

1975, *256*, 495. (b) Enguall, E. *Methods Enzymol.* **1980**, 70, 419.
(21) (a) Bednarski, M.D.; Chenault, H.K.; Simon, E.S.; Whitesides, G.M. *J. Am. Chem. Soc.* **1987**, *109*, 1283. (b) Wong, C.-H. *Science* **1989**, *244*, 1145.
(22) Trost, B.M. *Science* **1991**, *254*, 1471.
(23) Carsen, P.H.J.; Hatsuki, T.; Martin, V.S.; Sharpless, K.B. *J. Org. Chem.* **1981**, *46*, 3936.
(24) Dale, J.A.; Dull, D.L.; Mosher, H.S. *J. Org. Chem.* **1969**, *34*, 2543.
(25) Corey, E.J.; Fuchs, P.L. *Tetrahedron Lett.* **1972**, *3769*.
(26) Anderson, R.J.; Corbin, V.L.; Cotterrell, G.; Cox, G.R.; Henrick, C.A.; Schaub, F.; Siddall, J.B. *J. Am. Chem. Soc.* **1975**, *97*, 1197.
(27) Burgess, K.; Cassidy, J.; Ohlmeyer, M.J. *J. Org. Chem.* **1991**, *56*, 1020.
(28) The closest literature precedents involve enantioselective protonation of various metal enolates. See, for example: (a) Fehr, C.; Galindo, J. *J. Am. Chem. Soc.* **1988**, *110*, 6909. (b) Matsumoto, K.; Ohta, H. *Tetrahedron Lett.* **1991**, 32, *4729*. (c) Yasukata, T.; Koga, K. *Tetrahedron Asymmetry* **1993**, *4*, 35. (d) Vedejs, E.; Lee, N.; Sakata, S.T. *J. Am. Chem. Soc.* **1994**, *116*, 2175 and references cited therein.