Review Article

Asymmetric Organic Synthesis with Catalytic Antibodies*

Ehud Keinan,[†,a,b] Subhash C. Sinha,[a] Doron Shabat,[b] Harel Itzhaky[b] and Jean-Louis Reymond[a]

[a]The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037, USA and [b]Department of Chemistry, Technion-Israel Institute of Technology, Haifa 32000, Israel

Keinan, E., Sinha, S. C., Shabat, D., Itzhaky, H. and Reymond, J.-L., 1996. Asymmetric Organic Synthesis with Catalytic Antibodies. – Acta Chem. Scand. 50: 679–687. © Acta Chemica Scandinavica 1996.

The science of catalytic antibodies has undergone a rapid maturation process within its first nine years of existence. From initial 'proof of concept' and demonstration of fundamental, enzyme-like characteristics, antibodies have been shown to catalyze a remarkably broad scope of organic transformations, including difficult and unfavorable chemical reactions. Yet, the ultimate testing ground for new concepts in organic chemistry has always been the synthesis of natural products. Here we focus on several issues related to the applicability of antibody catalysis in organic synthesis. We show that (a) in the hydrophobic environment of the antibody active site, short-lived intermediates can be formed and reacted in a controlled way, thus allowing antibodies to catalyze reactions that are normally incompatible with aqueous media, (b) the intrinsic order of reactivity (chemoselectivity) in a series of structurally related enol ethers and ketals can be inverted from 1:10 in the uncatalyzed hydrolysis reaction to 1000:1 under antibody catalysis, and (c) an efficient total synthesis of α-multistriatin, an important, biologically active natural product can be achieved via antibody catalysis.

A. Antibody catalysis of a reaction otherwise strongly disfavored in water[1]

Many organic synthetic methods require the manipulation of highly reactive, water-sensitive intermediates. Consequently, the fact that catalytic antibodies[2] operate in water could render them useless catalysts for many organic transformations. We show here, however, that such chemistry is accessible using catalytic antibodies in aqueous media. These observations, which open interesting opportunities in synthesis, demonstrate the ability of antibody catalysis to promote reaction pathways that are normally disfavored.[3]

Oxocarbonium ion intermediates such as I (Fig. 1) and the glycosyl cation have lifetimes in water in the order of 10^{-5}–10^{-12} s.[4,5] In the acid-catalyzed hydrolysis of 1 the rate-determining step with the transition state of highest energy is the formation of intermediate I.

Partitioning of I to yield products 2 and 3 occurs after the rate-determining step and involves lower energy barriers,[6,7] as illustrated in the schematic potential energy surface of the reaction (Fig. 2). Thus, in order for a catalyst to promote ketal formation, it should be able both to lower the energy barrier between enol ether 1 and intermediate I, and to lower the energy barrier for conversion of I into ketal 3 relative to ketone 2.

Monoclonal antibody 14D9, which binds hapten 4a with high affinity, has been shown to catalyze several reactions involving oxocarbonium ion intermediates, including cleavage of a cyclic acetal,[8] and hydrolysis of ketals,[9] epoxides,[10] and enol ethers.[11] The latter reaction proceeds with high enantioselectivity of protonation at carbon and can be carried out on a preparative scale.[12] Catalysis occurs via stabilization of the intermediate oxocarbonium ion by an ionizable protein side chain.[11] A significant part of the binding energy between 14D9 and hapten 4a is provided by hydrophobic interactions with the methylpiperidinium moiety of the hapten, which implies that the antibody active site is highly hydrophobic.[13] We therefore expected that this antibody might

---

* Contribution presented at the Nobel Symposium on *Catalytic Asymmetric Synthesis*, September 3–7, 1995 at Tammsvik, Bro, Sweden.
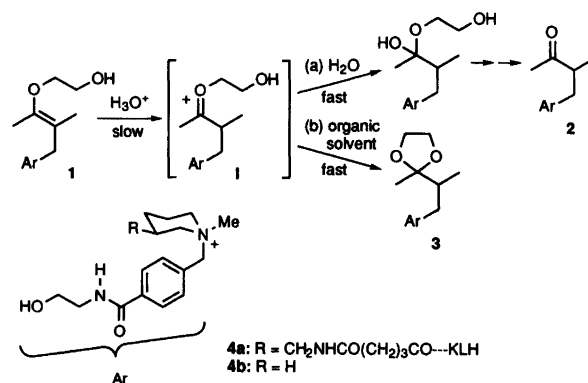† To whom correspondence should be addressed.

4a: R = CH₂NHCO(CH₂)₃CO---KLH
4b: R = H

Fig. 1. Oxocarbonium ion intermediate I is generated by protonation of enol ether **1** under acid catalysis. In aqueous media I is rapidly trapped by a molecule of water to yield a hemiketal and ultimately ketone **2** (pathway a). In organic solvents, under very low concentration of water, I can undergo ring closure to produce ketal **3** (pathway b).
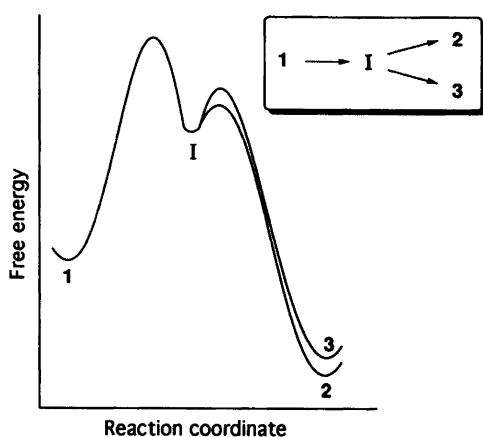

Fig. 2. Schematic diagram of the potential energy surface of the acid-catalyzed hydrolysis of **1**.
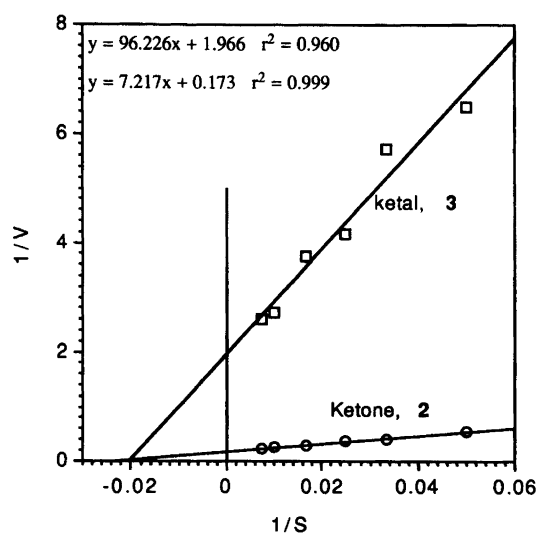

$y = 96.226x + 1.966 \quad r^2 = 0.960$
$y = 7.217x + 0.173 \quad r^2 = 0.999$

Fig. 3. A Lineweaver–Burke plot for the 14D9-catalyzed hydrolysis of **1** to **2** and **3**. Reactions were carried out in phosphate buffered saline, pH 6.06 at 25°C. Calculated K_m values for the formation of **2** and **3** are 42 and 50 μM, respectively. For the combined products: $k_{cat} = 1.2 \times 10^{-3}$, $k_{uncat} = 2.0 \times 10^{-6} \text{ s}^{-1}$.

not only catalyze the formation of intermediate I by protonation of enol ether **1**, but could also influence the partitioning step and lead to formation of ketal **3** by excluding water from the reaction center.

Acid-catalyzed hydrolysis of enol ether **1** in aqueous solution gives ketone **2** as the sole product. No traces of ketal **3** can be detected, indicating that the substrate's hydroxy group cannot compete against water on intermediate I. Antibody 14D9 catalyzes the hydrolysis of **1**. Catalysis is totally inhibited by hapten **4b** and follows saturation kinetics. Although the catalytic reactions are carried out in 100% aqueous medium, significant amounts of ketal **3** (approximately 12%) are observed in the reaction products. Formation of **2** and **3** follows Michaelis–Menten kinetics (Fig. 3), yielding comparable K_m values. This suggests that both the ketone and ketal products originate from a common intermediate that is formed catalytically in the antibody combining site.

The pH–rate profile of the hydrolysis of **1** was studied in either phosphate buffered saline or 1,3-bis[tris(hydroxymethyl)methylamino]propane buffered saline, with pH ranging from 5 to 9 (Fig. 4). The background reaction is first order in hydronium ion throughout the pH range. The antibody-catalyzed reac-

tion is first order in hydronium ion above pH 6 but shows saturation at lower pH. This behavior is in agreement with other reactions observed with this antibody, and indicates the participation of an ionizable side chain with a pK_a of 5.8, presumably a carboxy group, in catalysis.[10,11] Most significantly, the ratio between ketone **2** and ketal **3** formed in the antibody-catalyzed reaction is constant throughout the entire pH range. Similarly, this product distribution is independent of the reaction temperature between 4 and 37 °C. These observations suggest that the mechanistic pathways leading from intermediate I to either ketone or ketal are almost identical in terms of acid–base mechanism as well as thermodynamic activation parameters.
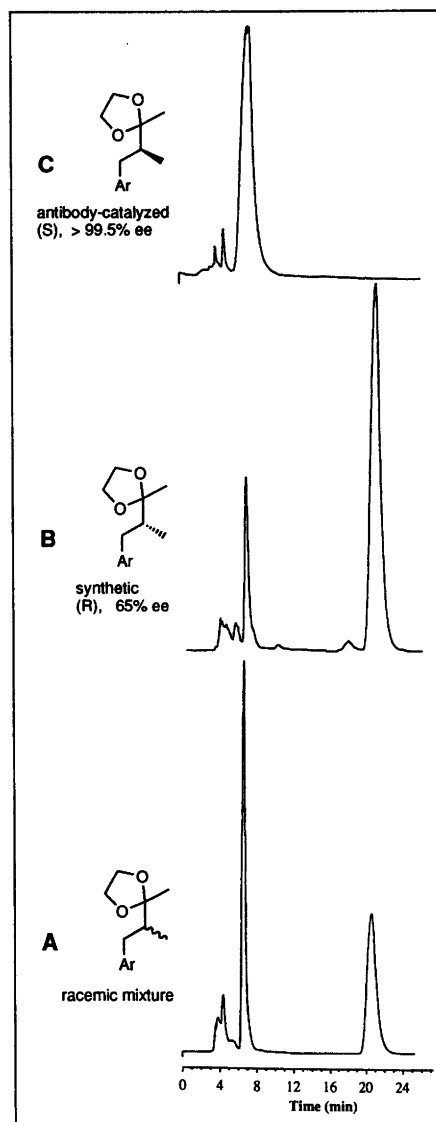
Both products **2** and **3** isolated from the same antibody-catalyzed reaction are obtained with high enantiomeric purity and with the same *S* absolute
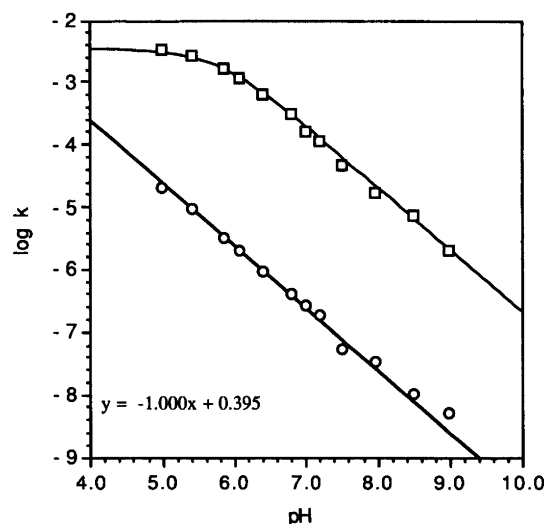
Fig. 4. pH–rate profile for the hydrolysis of 1. The upper data points (□) represent the experimental log k (s^{-1}) of the antibody 14D9-catalyzed reaction and the lower points (○) represent the hydronium-ion catalyzed reaction. Assays were carried out in either phosphate buffered saline or 1,3-bis[tris(hydroxymethyl)methylamino]propane buffered saline (50 mM buffer, 100 mM NaCl, pH 5.0–9.0) containing 1% DMF at 25°C using 3 μM antibody and 20–150 μM of 1. A double reciprocal plot of the experimental values $1/k_{cat}$ vs. $1/[H_3O^+]$ is linear (6 lower pH data points, $r^2 = 0.997$). This is consistent with the participation of an ionizable antibody side chain in catalysis, and gives the values $pK_a = 5.79$ for the dissociation constant of that side chain (probably either an aspartate or glutamate residue) and $(k_{cat})_{max} = 3.42 \times 10^{-3}$ s^{-1} for the maximum turnover number of the antibody at low pH. The calculated line was obtained by using these values in the following equation: $1/k_{cat} = 1/(k_{cat})_{max} + (1/[H_3O^+]) K/(k_{cat})_{max}$.[11] As there is no detectable pH-independent reaction, the background hydrolysis is a hydronium-ion catalyzed reaction, $k_{H^+} = 10^{0.395} = 2.48$ M^{-1} s^{-1}. The calculated line was obtained using these values in the following equation: $k_{uncat} = [H_3O^+]k_{H^+}$.

Fig. 5. Determination of absolute configuration and enantiomeric purity of ketal 3 produced in the antibody 14D9-catalyzed reaction of 1. All analyses were carried out by HPLC using a Chiracell OD-H column. A, HPLC chromatogram of a racemic mixture of 3. B, Chromatogram of (R)-3 synthesized from enantiomerically pure (1R,2S)-(−)-norephedrine using the Evans methodology.[26] C, Chromatogram of 3 produced by 14D9-catalyzed reaction.

configuration. The remarkably high enantiomeric purity of ketal 3 (>99.5% ee, Fig. 5) agrees with the fact that 3 is formed exclusively via antibody catalysis. Furthermore, it also represents the first direct proof that the rate-determining protonation at carbon is achieved with complete enantioselectivity in the antibody active site. These observations confirm the assumption that both ketal 3 and ketone 2 originate from partitioning of a single intermediate and not from two diastereoisomeric transition states, which could be formed by protonation at either of the prochiral faces of the double bond in 1.

To assess the difficulty of what has been accomplished here by antibody catalysis we have studied model systems. Formation of ketal 3 reflects the low availability of water molecules at the vicinity of the intermediate I in the antibody active site. Such a medium effect is expected to exist within the hydrophobic active site of antibody 14D9.[11,13] To test this hypothesis we used mixtures of either dioxane–water or isopropyl alcohol–water containing 5% acetic acid as a simple model environment.[14] Acetic acid was chosen to mimic the

residue responsible for general acid catalysis in the antibody active site. Measurable proportions of ketal are formed only at very low water concentration in acidic dioxane (Fig. 6). The situation is even worse in acidic isopropyl alcohol, where less than 1% ketal could be detected at water concentrations greater than 1%. The product distribution of the antibody reaction corresponds to that observed in acidic dioxane containing only 3% water.

Reaction rates drop by four orders of magnitude in these media as compared with pure water (Fig. 7). By contrast, the antibody accelerates the reaction by three
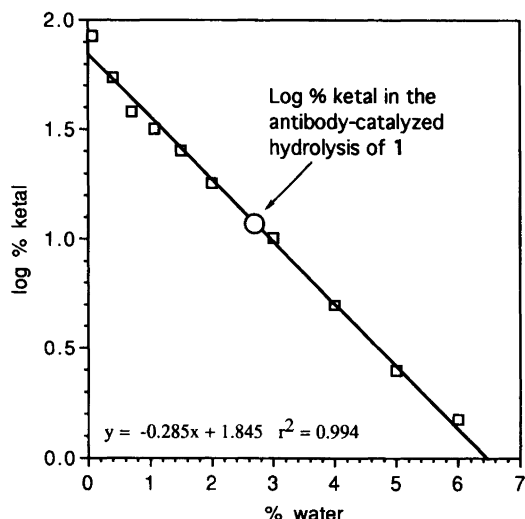
681

Fig. 6. Effect of water concentration on yields of ketal **3** in the uncatalyzed hydrolysis of **1**. All reactions were carried out at 24°C using varying mixtures of dioxane and water and a constant concentration (5% v/v) of acetic acid. The circular data point represents the 12% ketal formed in the antibody-catalyzed reaction. On this line it corresponds to 2.7% water in dioxane–acetic acid.
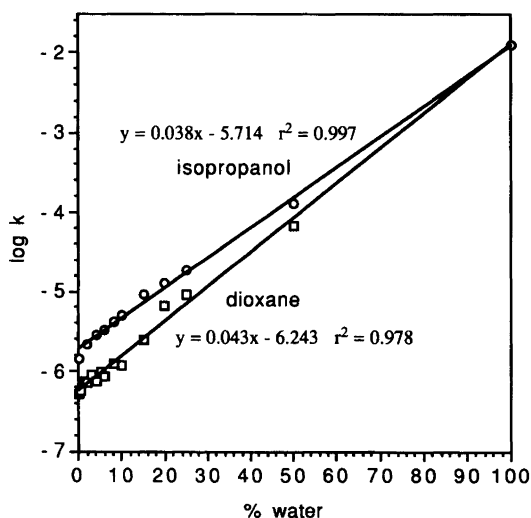

Fig. 7. Effect of water concentration on the rate of the acetic acid catalyzed hydrolysis of **1**. All reactions were carried out at 25°C with 10^{-4} M substrate **1**, using varying mixtures of either dioxane or isopropyl alcohol and water and a constant concentration (5% v/v) of acetic acid. The circular data points represent log of the rate constants (s^{-1}) of the reaction in isopropyl alcohol and the rectangular points represent the corresponding values of the dioxane reaction.

orders of magnitude. Attribution of ketal formation in the antibody active site to a medium effect would therefore lead to the conclusion that the antibody actually accelerates the reaction by seven orders of magnitude. Although the approximation of the antibody active site to a homogeneous, isotropic medium may be an over-

simplification, this model is consistent with the observation of constant ketal:ketone ratios over broad ranges of pH values and temperatures.

A very similar dependence of ketal proportions on water concentration is observed when perchloric acid $(5 \times 10^{-4}$ M) is employed as catalyst instead of acetic acid in either dioxane or isopropyl alcohol (both solutions, 5% acetic acid in water and 5×10^{-4} M perchloric acid in water have pH 3.5), clearly showing that the partitioning is independent of the acid catalyst used. Similarly, reaction rates with $HClO_4$ decline by four orders of magnitude with decreased water concentration (from 100 to 5%). However, rates are increased steeply by one order of magnitude at lower concentration of water in both solvents (Fig. 8). This rate increase reflects enhanced reactivity of the hydronium ion as it becomes solvated by cosolvents which are less basic than water. This superacidity effect is not observed with acetic acid because acetate is more basic than water and therefore buffers the reactivity of the proton. Although the ionizable side chain responsible for catalysis in the antibody active site $(pK_a = 5.8)^{[10,11]}$ should buffer the reactivity of the hydronium ion, a similar superacidity effect might explain part of the unusual rate enhancement observed with this catalyst.

As to the origin of ketal formation in the water–dioxane system, it is probably a dual effect of both lowered concentration of water molecules and increased alcohol nucleophilicity due to desolvation. While the same factors could operate in the antibody active site as
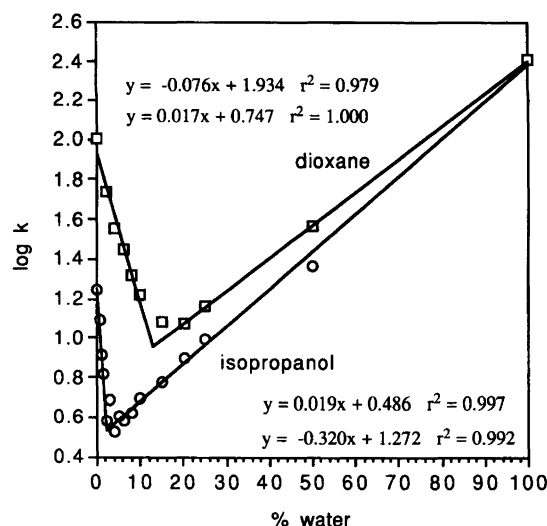

Fig. 8. Effect of water concentration on the rate of the perchloric acid catalyzed hydrolysis of **1**. All reactions were carried out at 25°C with 10^{-4} M substrate **1**, using varying mixtures of either dioxane or isopropyl alcohol and water and a constant concentration $(5 \times 10^{-4}$ M) of $HClO_4$. The circular data points represent log of the rate constants (s^{-1}) of the isopropyl alcohol reaction and the rectangular points represent the corresponding values of the dioxane reaction.

well, one should also consider the highly organized, asymmetric environment within that site which may restrict the available conformations of intermediate I and thereby control its partitioning.

One of the greatest advantages of natural enzymes is their ability to handle reactive intermediates under ambient temperatures in the presence of water. For example, formation of a glycosidic bond via organic synthesis requires strictly anhydrous conditions in order to prevent hydrolysis of the highly reactive glycosyl cation. Glycosyl transferases, however, catalyze the same reactions in water.[15] We have shown here that it is possible to generate and handle water–incompatible, highly reactive intermediates within the active site of a catalytic antibody in aqueous solution. This has been demonstrated by a highly difficult chemical task of ketal formation in water. The isolation of ketal 3 in very high enantiomeric purity is a striking example of the potential utility of such chemistry. This proves that the 14D9-catalyzed protonation at carbon is absolutely enantioselective. A wide array of synthetic transformations involving other water-incompatible, reactive intermediates should now be considered for antibody catalysis.

B. Antibody-catalyzed reversal of chemoselectivity[9]

The essence of chemical catalysis is the ability not only to enhance the rate of a given reaction but also to achieve chemoselectivity, regioselectivity and stereochemical control. It is now well established that reactions catalyzed by antibodies are likely to exhibit high levels of regio- and stereo-selectivity.[2] Chemoselectivity, and particularly reversal of chemoselectivity, is difficult to achieve because it involves selection of one out of several, nearly equivalent reaction coordinates. It involves modification of the intrinsic order of reactivity in a given series of functional groups. In most cases where reversal of chemoselectivity has been achieved it involved change in mechanism. A classical example compares the relative reactivity of a primary bromide and an allylic acetate towards displacement by carbon nucleophiles. The allylic acetate, which is normally less reactive becomes much more reactive when the reaction is catalyzed by Pd(0).[16]

Here we report on a remarkable case of chemoselectivity control by an antibody capable of catalyzing the hydrolysis of structurally similar ketals and enol ethers, all of which are shown to bind to the antibody active site with comparable affinity. The enol ether and ketal derivatives of a given ketone exhibit almost identical reactivity toward hydronium ion catalyzed hydrolysis in water. Chemoselective activation of the ketal function in preference to the enol ether can be achieved with Lewis acids in anhydrous media.[17] In turn, the enol ether function can be selectively hydrolyzed in the presence of a ketal using weak, general acids in water. Herein we

report upon an antibody that modifies these reactivity patterns in an unprecedented manner. This antibody (14D9) catalyzes the hydrolysis of the enol ethers of a ketone in the presence of its ketal derivative. Nevertheless, the same antibody catalyzes the hydrolysis of other closely related ketals. The inversion of the reactivity order within a series of ketal substrates under antibody catalysis represents an unprecedented reversal of chemoselectivity.

Hydrolysis of both enol ethers 5 and 6 (Fig. 9), which proceeds via the same intermediate II, is catalyzed by antibody 14D9. Under acid catalyzed hydrolysis the related ketal 7 probably shares the same oxocarbonium intermediate II. As hydrolyses of both enol ethers and ketals are known to have late transition states,[18] we expected that 7 should be a suitable substrate for antibody 14D9. Nevertheless, despite the fact that the rate of hydrolysis of 7 in water is greater than that of 5 and 6, no measurable catalysis could be detected with this ketal (Fig. 9). The order of reactivity of 5, 6 and 7 has dramatically changed from 0.09:0.17:1 (relative rates for spontaneous hydrolysis at 20 °C, pH 7.0) to 1100:25:1 (under antibody catalysis).

In contrast with the behavior of 7, the two less rigid ketals 9 and 10 are catalytically hydrolyzed by 14D9 (Table 1). This enzyme-like catalysis is evident from the observed Michaelis–Menten kinetics and from the fact that catalysis is totally inhibited in the presence of stoichiometric quantities (with respect to 14D9) of the inhibitor 4b. At 0°C and pH 7.55, the order of reactivity of the three chemically similar ketals, 9, 10 and 7 toward hydrolysis is 0.23:0.38:1, respectively. However, this order is reversed to 90:8:1 within the antibody active site. Remarkably, all three ketals show very similar affinity to 14D9. The K_m values of substrates 9 and 10 are comparable (Table 1). Moreover, ketal 7, which shows no measurable catalysis, acts as a competitive inhibitor of 14D9-catalyzed hydrolysis of 9 with

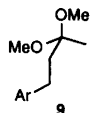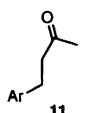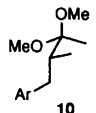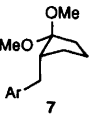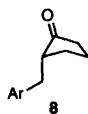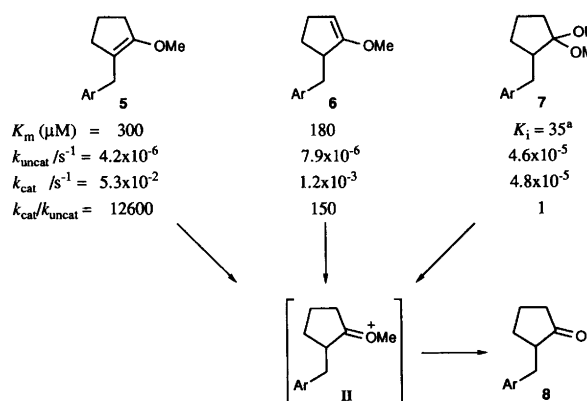|		
K_m (μM) = 300	180	K_i = 35[a]
k_{uncat} /s^{-1} = 4.2x10^{-6}	7.9x10^{-6}	4.6x10^{-5}
k_{cat} /s^{-1} = 5.3x10^{-2}	1.2x10^{-3}	4.8x10^{-5}
k_{cat}/k_{uncat} = 12600	150	1

Fig. 9. Hydrolysis of enol ethers 5 and 6 and ketal 7 to ketone 8. All reactions were carried out in 50 mM phosphate buffer and 100 mM NaCl at pH 7.55 and 0°C. ^aK_i = inhibition constant for competitive inhibition of the 14D9-catalyzed hydrolysis of 9.
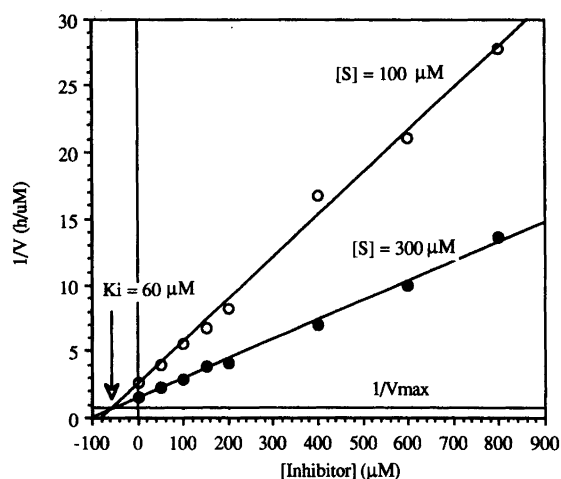
Table 1. Kinetic data for 14D9-catalyzed hydrolysis of ketals 7, 9 and 10. All reactions were carried out in 50 mM phosphate buffer and 100 mM NaCl at pH 7.55 and 0°C.

Substrate	Product	$K_m/\mu m$	k_{uncat}/s^{-1}	k_{cat}/s^{-1}	$k_{cat}/_{uncat}$
9	**11**	230	4.2×10^{-7}	1.8×10^{-4}	430
10	**12**	160, $K_i = 60^a$	6.9×10^{-7}	1.6×10^{-5}	23
7	**8**	$K_i = 35^a$	1.8×10^{-6}	2×10^{-6}	1^b

^{a}Inhibition constants for competitive inhibition of the 14D9-catalyzed hydrolysis of **9**. ^{b}No rate enhancement could be detected.

$K_i = 35$ μM. The corresponding K_i value for **10** is 60 μM (Fig. 10).

The hydrolysis of enol ethers, such as **5** and **6**, involves proton transfer from the acid catalyst to the carbon atom in the rate determining step, and is catalyzed by general acids (mechanism b in Fig. 11).[19] By contrast, hydrolysis of ketals, such as **7**, proceeds by a rapid pre-equilibrium protonation of the ketal oxygen followed by rate-determining heterolysis of the carbon–oxygen bond (specific acid catalysis, mechanism a in Fig. 11). Thus, the hydrolysis rates of **7**, **9** and **10** are not affected by general acids and depend on the pH only. Accordingly, the selectivity of antibody 14D9 for **5** and **6** over **7** could be attributed to the presence of a protein side chain

Fig. 10. Inhibition of 14D9-catalyzed hydrolysis of **9** with ketal **10**. All reactions were carried out in 50 mM phosphate buffer and 100 mM NaCl at pH 7.55 and 0°C. [S] is the concentration of **9** and [Inhibitor] is the concentration of **10**.

acting as a general acid in the active site. However, the observation of a catalytic reaction with ketals **9** and **10** clearly demonstrates that 14D9 can hydrolyze ketals closely related to **7** despite the fact that these compounds are totally unreactive towards general acids. A large inverse kinetic solvent isotope effect is observed for the 14D9-catalyzed hydrolysis of ketal **9**: $(k_D/k_H)_{cat} = 2.4$. This value is similar to the isotope effect for the uncatalyzed reaction with hydronium ion: $(k_D/k_H)_{uncat} = 2.5$. These values are consistent with specific acid catalysis (mechanism a in Fig. 11). A similarly large, inverse solvent isotope effect $(k_D/k_H = 2.82)$ has been reported for the hydronium-ion-catalyzed hydrolysis of 2-ethoxytetrahydropyran.[20] General acid catalysis in acetal/ketal hydrolysis has been observed either in cases where the leaving group is highly reactive, e.g., with 2-(p-nitrophenoxy)tetrahydropyran, where $k_D/k_H = 1.33$ was measured for the hydronium-ion catalysis,[20] or when the oxocarbonium intermediate is highly stable, e.g., in the case of tropone diethyl ketal.[18b] In the two special cases mentioned above, a significant pH-independent hydrolysis was also observed, in conjunction with general acid catalysis. We measured the pH–rate profile for the background hydrolysis of ketals **7** and **9** between pH 6.0 and 10.0 (50 mM bistrispropane, 100 mM NaCl) and found that hydrolysis rates of both compounds are proportional to the hydronium ion concentration [slope of -1 for $\log(k_{uncat})$ vs. pH] throughout the entire range, with no detectable pH-independent component. This reinforces previous observations that our ketal substrates, despite their high hydrolysis rates in water, display normal reactivity and undergo hydrolysis under specific acid catalysis only.

As the lack of reactivity of **7** cannot be attributed to a simple chemical effect (specific vs. general acid catalysis) or to insufficient binding to the antibody, we propose that the origin of this phenomenon is related to conformational effects within the antibody binding site. The binding of hapten **4a**, inhibitor **4b** as well as compounds **6–10** is primarily mediated by recognition of the common substituted benzene moiety. We have shown previously that the antibody binding pocket is complementary to hapten **4a** in the chair conformation, with the benzylic substituent taking an axial position, and that close hydrophobic contacts exist between the antibody and the methylpiperidinium moiety of the hapten.[13] This would imply that in the bound state, the different substrates adopt a conformation closely related to that of **4a**. The lack of reactivity of **7** can be explained if one assumes that, in the antibody–substrate complex, the rate determining departure of methanol has to proceed along a trajectory that goes deep into the hydrophobic pocket (**III**). Both **9** and **10** can reach the reactive conformation by rotation around the homobenzylic C–C bond, but not the rotationally restricted, cyclic substrate **7**. The reactivity of enol ethers **5** and **6** relative to **7** would suggest that this type of stereochemical constraint does not apply for protonation of their double bonds.

III R^1, R^2 = Me, OMe
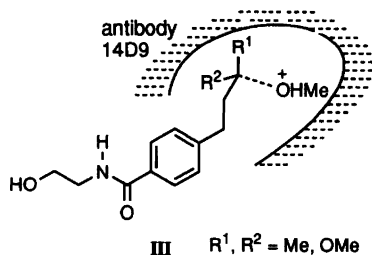
In conclusion, the ability to control chemoselectivity by chemical catalysis represents one of the chemist's main goals. We present here two examples of reversal of chemoselectivity that were achieved under antibody catalysis: (a) activation of enol ethers in the presence of a ketal and (b) inversion of the order of reactivity in structurally similar ketals. Since all compounds involved in this study bind the antibody with very similar affinities and are structurally related, these effects cannot be simply attributed to selective binding by the antibody or to the placement of the reactive functionality away from the catalytically active site. The observed chemoselectivity might reflect conformational constraints on the ketal cleavage process within the antibody binding site. These observations may prove useful in the future design of selective biocatalysts.

C. Asymmetric synthesis of (−)-α-multistriatin via antibody catalysis[21]

(−)-α-Multistriatin, **13**, is an essential component of the aggregation pheromone of the European elm bark beetle, *Scolytus multistriatus* (Marsham), which is the principal vector of Dutch elm disease.[22] The severe devastation of the elm population in Northeastern USA has resulted in extensive studies of the synthesis[23] and field utilization. Field experiments show that the inactive, (+)-enantiomer of **13** inhibits the biological activity of the naturally occurring (−)-enantiomer[24,25] of **13**. In order to achieve the required absolute configuration in all four asymmetric centers (1S,2R,4S,5R), most of the previous syntheses of **13** employ enantiomerically pure natural products as starting materials.[23] We focused on the opportunity to

achieve the required chirality via asymmetric synthesis of ketone, **14**, using antibody catalysis. As discussed above, monoclonal antibody 14D9 has already been proved to be an effective catalyst for hydrolysis of a variety of substrates that are structurally related to **14** (Fig. 12), including ketals,[9] epoxides,[10] and enol ethers.[1,11–13] One might therefore expect that ketone **14** would be readily available by antibody-catalyzed enantioselective protonolysis of the corresponding isomeric enol ethers **15a** (Z) and **15b** (E). This antibody-catalyzed step could be the only source of asymmetry in the molecule, provided that the following chemical steps proceed with control over the relative stereochemistry.

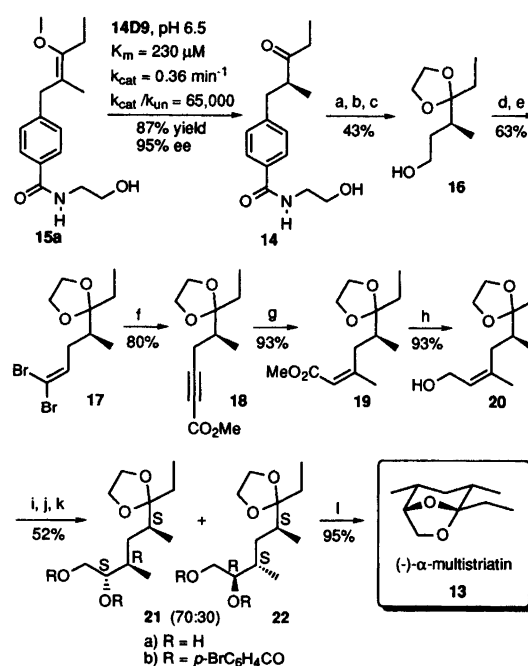In acidic, aqueous media, both isomers are hydrolyzed

Fig. 12. Stereoselective synthesis of α-multistriatin. Key: a, RuCl₃, NaIO₄, CCl₄, CH₃CN, H₂O; b, Ethylene glycol, PPTS, benzene; c, LiAlH₄, ether; d, PCC, CH₂Cl₂; e, CBr₄, PPh₃ CH₂Cl₂; f, n-BuLi, NCCO₂Me, THF; g, MeCu, TMEDA, ether; h, DIBAL-H, THF, toluene; i, BH₃·SMe₂, then H₂O₂/NaOH; j, p-BrC₆H₄COCl, Et₃N, DMAP, CH₂Cl₂, column chromatography; k, LiAlH₄, ether; l, PPTS, CH₂Cl₂.
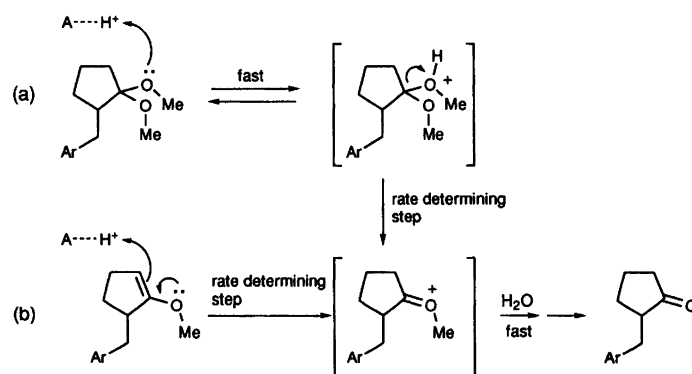

Fig. 11. Mechanistic pathways of the hydrolysis of enol ethers **5** and **6** and ketal **7** to ketone **8**.

685

to racemic ketone **14** at a comparable rate. Antibody 14D9 catalyzes this reaction under mildly acidic conditions (Fig. 12). Interestingly, catalysis with the Z enol ether **15a** is much more effective ($k_{cat}/k_{un} = 65\,000$) than with the E isomer, **15b**, ($k_{cat}/k_{un} = 5\,000$). This enzyme-like catalysis is evident from the observed Michaelis–Menten kinetics (**15a**: $K_m = 230$ mM, $k_{cat} = 0.36$ min^{-1} at pH 6.5; **15b**: $K_m = 310$ µM, $k_{cat} = 0.044$ min^{-1} at pH 6.0) and from the fact that catalysis is totally inhibited in the presence of stoichiometric quantities (with respect to 14D9) of the methylpiperidinium hapten **4b**. Both **15a** and **15b** are hydrolyzed by 14D9 to produce ketone **2** with the same absolute configuration (S), probably due to the structural similarity between both substrates. Determination of absolute configuration and enantiomeric purity of ketone **2** was carried out by reduction with NaBH$_4$ to the corresponding diastereomeric mixture of alcohols, followed by HPLC analysis using a Chiracell OD-H column. The absolute configuration was determined by comparison with an authentic sample synthesized from enantiomerically pure (1S,2R)-(+)-norephedrine using the Evans methodology,[26] which is known to proceed with high diastereoselectivity and predictable absolute configuration. The high rate acceleration in the case of **15a** allows the reaction to be driven to near completion to produce **14** in greater than 99% ee.

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. Efficient catalysis is thus achieved with a partially purified 14D9 which was precipitated from the ascites fluid by saturated ammonium sulfate (SAS). We carried out the antibody-catalyzed reaction on a preparative scale using very simple organic-laboratory equipment using dialysis bags.[12,27] In each catalytic cycle a solution of the enol ether **15a** (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 relatively lower solubility of the starting material relative to that of the product) and became clear as the reaction reached completion. Recovery of the catalyst after each cycle was achieved using cellulose dialysis bags (allowing diffusion of molecules smaller than 12–14 kDa). The reaction was interrupted after 60 h at 80% conversion by transferring the mixture into a dialysis bag and dialyzed into 500 ml of the same buffer over 16 h. The antibody solution was taken to the next catalytic cycle with a fresh solution of **15a**. The buffer solution was saturated with sodium chloride and extracted with dichloromethane. 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. In this large-scale reaction the enantiomeric purity of the prod-

uct **14** (95% ee, 87% yield) was found to be somewhat lower than that observed in the small-scale preparation (99% ee, 95% yield).

Ketone **14** was converted into **13** in a sequence of twelve chemical reactions (Fig. 12). The aromatic portion of **14** was exhaustively degraded with RuCl$_3$ and sodium periodate.[28] The resultant keto acid was then converted into a ketal alcohol **16** by reaction with ethylene glycol and catalytic amounts of pyridinium p-toluenesulfonate (PPTS) in benzene followed by reduction with LiAlH$_4$ in ether. An NMR spectrum of the Mosher ester[29] of **16** indicated 92% ee, reflecting only minor loss of enantiomeric purity throughout these three steps. Alcohol **16** 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, **17**.[30] Treatment of **17** with n-butyllithium and methyl cyanoformate produced the substituted methyl propargylate **18**. Reaction of the latter with 'MeCu' (prepared from methyllithium and CuI in THF and tetramethylethylenediamine) afforded geometrically pure (Z)-α,β-unsaturated ester **19**.[31] Reduction of this ester with diisobutylaluminum hydride in toluene–THF afforded the corresponding allylic alcohol, **20**, with retention of the Z geometry. Treatment of **20** with a solution of borane–dimethyl sulfide complex in THF followed by oxidation with basic (NaOH, 3 M) hydrogen peroxide produced a 70:30 mixture of two diastereomeric products: **21** (having the required 4S, 6R, 7S configuration) and its 4S,6S,7R-diastereomer, **22**, respectively. Esterification with 4-bromobenzoyl chloride produced the corresponding bis-bromobenzoate derivatives which were easily separated by column chromatography. The purified diol **21** was then recovered via reductive cleavage with LiAlH$_4$. Finally, treatment of **21** with catalytic amounts of pyridinium p-toluenesulfonate in dichloromethane followed by Kugelrohr distillation at 110°C afforded (−)-α-multistriatin, **13** in the form of a colorless oil. The synthetic pheromone **13** has been checked in field experiments and found to be as active as the naturally occurring compound in attracting the European-elm bark-beetles into traps loaded with a mixture of **13** with (−)-α-cubebene (a host-produced component) and (−)-4-methylheptan-3-ol.

In 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.[32]

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 dec-

ades. 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.

References

1. Shabat, D., Itzhaky, H., Reymond, J.-L. and Keinan, E. *Nature (London) 374* (1995) 143.
2. (a) Lerner, R. A., Benkovic, S. J. and Schultz, P. G. *Science 252* (1991) 659; (b) Schultz, P. G. and Lerner, R. A. *Science 269* (1995) 1835.
3. Schultz, P. G. and Lerner, R. A. *Acc. Chem. Res. 26* (1993) 391.
4. Banait, N. S. and Jencks, W. P. *J. Am. Chem. Soc. 113* (1991) 7591.
5. Pothier, N., Goldstein, S. and Deslongchamps, P. *Helv. Chim. Acta 75* (1992) 604.
6. Jenks, W. P. *Catalysis in Chemistry and Enzymology*, Dover Publications, New York 1987, pp. 52–60.
7. Schmir, G. L. and Gunningham, B. A. *J. Am. Chem. Soc. 87* (1965) 5692.
8. Reymond, J.-L., Janda, K. D. and Lerner, R. A. *Angew. Chem., Int. Ed. Engl. 30* (1991) 1711.
9. Sinha, S. C., Keinan, E. and Reymond, J.-L. *Proc. Natl. Acad. Sci. USA 90* (1993) 11910.
10. Sinha, S. C., Keinan, E. and Reymond, J.-L. *J. Am. Chem. Soc. 115* (1993) 4893.
11. Reymond, J.-L., Jahangiri, G. K., Stoudt, C. and Lerner, R. A. *J. Am. Chem. Soc. 115* (1993) 3909.
12. Reymond, J.-L., Reber, J.-L. and Lerner, R. A. *Angew. Chem., Int. Ed. Engl. 33* (1994) 475.
13. Jahangiri, G. K. and Reymond, J.-L. *J. Am. Chem. Soc. 166* (1994) 11264.
14. Lewis, C., Paneth, P., O'Leary, M. H. and Hilvert, D. *J. Am. Chem. Soc. 115* (1993) 1410.
15. Schuster, M., Wang, P., Paulson, J. C. and Wong, C.-H. *J. Am. Chem. Soc. 116* (1994) 1135.
16. Trost, B. M. *Acc. Chem. Res. 13* (1980) 385.
17. (a) Katritzky, A. R., Rachwal, S., Rachwal, B. and Steel, P. J. *J. Org. Chem. 57* (1992) 4925; (b) Faunce, J. A., Grisso, B. A. and Mackenzie, P. B. *J. Am. Chem. Soc. 113* (1991) 3418, and references cited therein.
18. (a) Kresge, A. J., Sagatys, D. S. and Chen, H. L. *J. Am. Chem. Soc. 99* (1977) 7228; (b) Anderson, E. and Fife,
T. H. *J. Am. Chem. Soc. 91* (1969) 7613; (c) Kirby, A. J. *CRC Crit. Rev. Biochem. 22* (1987) 283, and references cited therein.
19. Kresge, A. J., Chen, H. L., Chaing, Y., Murrill, E., Payne, M. A. and Sagatys, D. S. *J. Am. Chem. Soc. 93* (1971) 413.
20. Fife, T. H. and Jao, L. K. *J. Am. Chem. Soc. 90* (1968) 4081.
21. Sinha, S. C. and Keinan, E. *J. Am. Chem. Soc. 117* (1995) 3653.
22. (a) Lanier, G. N., Silverstein, R. M. and Peacock, J. W. In: Anderson, J. F and Kaya, H. K., Eds., *Perspectives of Forest Entomology*, Academic Press, New York, 1976, Chap. 12; (b) Pearce, G. T., Gore, W. E., Silverstein, R. M., Peacock, J. W., Cuthbert, P. A., Lanier, G. N. and Simeone, J. B. *J. Chem. Ecol. 1* (1975) 115.
23. (a) Gore, W.E., Pearce, G.T. and Silverstein, R.M. *J. Org. Chem. 40* (1975) 1705; (b) Pearce, G.T., Gore, W.E. and Silverstein, R.M. *J. Org. Chem. 41* (1976) 2797; (c) Elliot, W.J. and Fried, J. *J. Org. Chem. 41* (1976) 2475; (d) Mori, K. *Tetrahedron 32* (1976) 1979; (e) Cernigliaro, G.J. and Kocienski, P.J. *J. Org. Chem. 42* (1977) 3622; (f) Sum, P.-E. and Weiler, L. *Can. J. Chem. 56* (1978) 2700; (g) Elliot, W.J., Hromnak, G., Fried, J. and Lanier, G.N. *J. Chem. Ecol. 5* (1979) 269; (h) Bartlett, P.A. and Myerson, J. *J. Org. Chem. 44* (1979) 1625; (i) Fitzsimmons, B.J., Plaumann, D.E. and Fraser-Reid, B. *Tetrahedron Lett.* (1979) 3925; (j) Marino, J.P. and Abe, H. *J. Org. Chem. 46* (1981) 5379; (k) Sum, P.-E. and Weiler, L. *Can. J. Chem. 60* (1982) 327; (l) Plaumann, D.E., Fitzsimmons, B.J., Ritchie, B.M. and Fraser-Reid, B. *J. Org. Chem. 47* (1982) 941; (m) Walba, D.M. and Wand, M.D. *Tetrahedron Lett. 23* (1982) 4995; (n) Langrange, A., Olesker, A., Soares Costa, S., Lukacs, G. and Thang, T.T. *Carbohydr. Res. 110* (1982) 159; (o) Helbig, W. *Liebigs Ann. Chem.* (1984) 1165; (p) Larcheveque, M. and Henrot, S. *Tetrahedron 43* (1987) 2303; (q) Mori, K. and Seu, Y.-B. *Tetrahedron 44* (1988) 1035.
24. Lanier, G.N., Gore, W.E., Pearce, G.T., Peacock, J.W. and Silverstein, R.M. *J. Chem. Ecol. 3* (1988) 1.
25. Elliott, W.J., Hromnak, G., Fried, J. and Lanier, G.N. *J. Chem. Ecol. 5* (1979) 279.
26. Evans, D.A., Ennis, M.D. and Mathre, D.J. *J. Am. Chem. Soc. 104* (1982) 1737.
27. Bednarski, M.D., Chenault, H.K., Simon, E.S. and Whitesides, G.M. *J. Am. Chem. Soc. 109* (1987) 1283.
28. Carsen, P.H.J., Hatsuki, T., Martin, V.S. and Sharpless, K.B. *J. Org. Chem. 46* (1981) 3939.
29. Dale, J.A., Dull, D.L. and Mosher, H.S. *J. Org. Chem. 34* (1969) 2453.
30. Corey, E.J. and Fuchs, P.L. *Tetrahedron Lett.* (1972) 3769.
31. Anderson, R.J., Corbin, V.L., Cotterrell, G., Cox, G.R., Henrick, C.A., Schaub, F. and Siddall, J.B. *J. Am. Chem. Soc. 97* (1975) 1197.
32. (a) Fehr, C. and Galindo, J. *J. Am. Chem. Soc. 110* (1988) 6909; (b) Matsumoto, K. and Ohta, H. *Tetrahedron Lett. 32* (1991) 4729; (c) Yasukata, T. and Koga, K. *Tetrahedron Asymmetry 4* (1993) 35; (d) Vedejs, E., Lee, N. and Sakata, S.T. *J. Am. Chem. Soc. 116* (1994) 2175.