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# Antibody-catalyzed reversal of chemoselectivity

(catalytic antibodies/ketals/enol ethers/hydrolysis)

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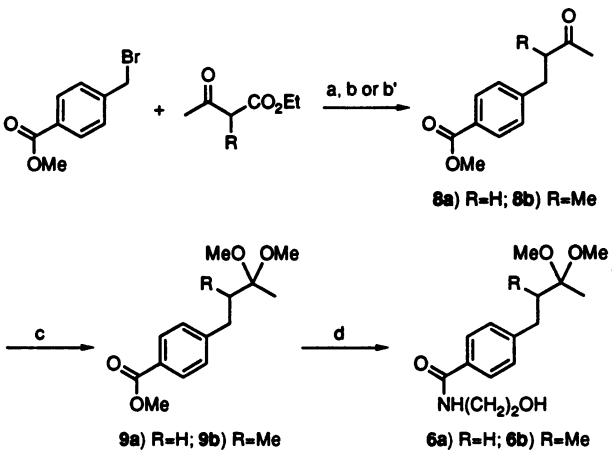
**ABSTRACT** **A monoclonal antibody, 14D9, which has been elicited against a cationic hapten, *N*-alkyl-*N*-methyl-3-glutarylamidomethyl piperidinium, in which alkyl = [4-(2-hydroxyethylamido)carbonyl]phenylmethyl, is capable of inverting the intrinsic order of reactivity in a series of structurally related enol ethers and ketals towards hydrolysis. The order of reactivity of compounds 2 (1-methoxy-2-alkylcyclopent-1-ene), 3 (1-methoxy-5-alkylcylopent-1-ene), and 4 (1,1-dimethoxy-2-alkylcyclopentane) has changed from 0.09:0.17:1 in the uncatalyzed reaction to 1100:25:1 under antibody catalysis. Also, the order of reactivity of the three chemically similar ketals, 6a (1-alkyl-2,2-dimethoxypropane), 6b (1-alkyl-1-methyl-2,2-dimethoxypropane), and 4, has changed from 0.23:0.38:1 in the uncatalyzed hydrolysis to 100:9:1 within the antibody active site. As all compounds bind the antibody with very similar affinities, these effects cannot be simply attributed to selective binding by the antibody. In fact, ketal 4, which shows no measurable catalysis, acts as a competitive inhibitor of 14D9-catalyzed hydrolysis of 6a. Both the solution and the antibody-catalyzed hydrolysis of the ketal substrates are shown to be specific acid catalyzed, involving the unimolecular cleavage of the protonated substrate or antibody–substrate complex in the rate-determining step. Reactivity effects from the acid catalyst itself on ketal hydrolysis (reagent-controlled reactivity) are ruled out under this mechanistic scheme.**

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 stereoselectivity (1). Chemoselectivity is particularly difficult to control because it involves selection of one out of several nearly equivalent reaction coordinates. An interesting example is the antibody-catalyzed chemoselective reduction of a diketone to a single enantiomer of hydroxyketone, which is essentially impossible to achieve by known chemical methods (2, ‡).

We now 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 (refs. 4 and 5 and references cited in ref. 5). In turn, the enol ether function can be selectively hydrolyzed in the presence of a ketal by weak general acids in water. Herein we report upon an antibody that modifies these reactivity patterns in an unprecedented manner. This antibody 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 is an unprecedented reversal of chemoselectivity.§

## MATERIALS AND METHODS

**Preparation of Substrates.** Enol ethers **2** and **3** as well as ketal **4** have been prepared by procedures described earlier (7). Ketals **6a** and **6b** were synthesized as shown in Scheme I:




Scheme I

*2,2-Dimethoxy-4-[4-(2-hydroxyethylamido)carbonyl]phenylbutane* (**6a**). A mixture of methyl (4-bromomethyl)benzoate (1.15 g, 5 mmol), ethyl acetoacetate (1.95 g, 15 mmol), $K_2CO_3$ (829 mg, 6 mmol) in 19:1 acetone/dimethylformamide (20 ml) was refluxed for 12 h (step a). The solvent was removed under reduced pressure and the residue was dissolved in diethyl ether and washed with water. The organic layer was concentrated and dissolved in dimethyl sulfoxide (10 ml). Saturated aqueous NaCl (1 ml) was added and the

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‡We have reported on an alcohol dehydrogenase from *Thermoanaerobium brockii* that catalyzes the same type of transformation (3). As in the antibody-catalyzed reaction, the chemoselection reflects different binding affinities between the biocatalyst and the two carbonyl functions.

§Reversal of chemoselectivity is a particularly intriguing challenge, as it involves modification of the intrinsic order of reactivity in a given series of functional groups. In some of the rare cases where reversal of chemoselectivity was 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) (6).

mixture was refluxed for 6 h (step b), then mixed with water, extracted with ether, and purified by column chromatography (silica gel, hexane/ethyl acetate, 9:1, vol/vol) to give 4-(4-methoxycarbonyl)phenylbutan-2-one, **8a** (620 mg, 60%). $^1$H NMR ($C^2HCl_3$, 400 MHz): $\delta$ 7.95 (d, $J$ = 8.0 Hz, 2H), 7.25 (d, $J$ = 8.0 Hz, 2H), 3.90 (s, 3H), 2.95 (t, $J$ = 7.6 Hz, 2H), 2.78 (t, $J$ = 7.6 Hz, 2H), 2.15 (s, 3H).

A solution of **8a** (206 mg, 1 mmol), *p*-toluenesulfonic acid (5 mg) in trimethyl orthoformate (5 ml), and methanol (5 ml) was stirred at room temperature for 2 h (step c). Saturated aqueous sodium bicarbonate was added and the mixture was extracted with dichloromethane to give 2,2-dimethoxy-4-(4-methoxycarbonyl)phenylbutane, **9a**, which was taken to next step without further purification. $^1$H NMR ($C^2HCl_3$): 7.96 (d, $J$ = 8.4 Hz, 2H), 7.27 (d, $J$ = 8.4 Hz, 2H), 3.90 (s, 3H), 3.21 (s, 6H), 2.70 (m, 2H), 1.93 (m, 2H), 1.35 (s, 3H).

The crude **9a** described above was dissolved in ethanolamine (1 ml) and stirred at 60°C for 16 h (step d), then worked up with water and dichloromethane, and the organic layer was passed through a short bed of silica gel with ethyl acetate to give **6a** (180 mg, 63% from **8a**). $^1$H NMR ($C^2HCl_3$): 7.69 (d, $J$ = 8.2 Hz, 2H), 7.22 (d, $J$ = 8.2 Hz, 2H), 6.90 (br t, $J$ = 5.3 Hz, 1H), 3.78 (t, $J$ = 4.8 Hz, 2H), 3.57 (q, $J$ = 4.8 Hz, 2H), 3.20 (s, 6H), 2.66 (m, 2H), 1.89 (m, 2H), 1.34 (s, 3H).

*2,2-Dimethoxy-3-methyl-4-[4-(2-hydroxyethylamido)carbonyl]phenylbutane* (**6b**). Methyl ethyl acetoacetate (2.16 g, 15 mmol) was allowed to react with methyl (4-bromomethyl)benzoate (1.15 g, 5 mmol) as described above. The crude product was added to a mixture of HBr (48%, 10 ml) and conc. $H_2SO_4$ (2 ml), refluxed for 2 h, and then worked up with water and ether. The organic layer was concentrated, dissolved in methanol (10 ml) and conc. $H_2SO_4$ (0.2 ml), and refluxed for 16 h (step b′ in Scheme I). Solvents were removed under reduced pressure, water was added, and the mixture was extracted with ether. Chromatographic purification (silica gel, hexane/ethyl acetate, 9:1) afforded 3-methyl-4-(4-methoxycarbonyl)phenylbutan-2-one, **8b** (605 mg, 55%). $^1$H NMR ($C^2HCl_3$, 400 MHz): 7.95 (d, $J$ = 8.4 Hz, 2H), 7.22 (d, $J$ = 8.4 Hz, 2H), 3.89 (s, 3H), 3.05 (dd, $J$ = 13.6, 6.9 Hz, 1H), 2.84 (m, 1H), 2.61 (dd, $J$ = 13.6, 7.6 Hz, 1H), 2.10 (s, 3H), 1.09 (d, $J$ = 7.0 Hz, 3H).

Ketone **8b** (220 mg, 1 mmol) was converted to 2,2-dimethoxy-3-methyl-4-(4-methoxycarbonyl)phenylbutane, **9b**, by using the procedure described above. $^1$H NMR: 7.95 (d, $J$ = 8.2 Hz, 2H), 7.25 (d, $J$ = 8.2 Hz, 2H), 3.90 (s, 3H), 3.27 (s, 3H), 3.18 (s, 3H), 3.06 (d, $J$ = 12.4 Hz, 1H), 2.17 (q, $J$ = 11.5 Hz, 1H), 2.13 (m, 1H), 1.23 (s, 3H), 0.79 (d, $J$ = 6.6 Hz, 3H). Compound **9b** was finally converted to **6b** as described above (175 mg, 60% from **8b**). $^1$H NMR: 7.70 (d, $J$ = 8.0 Hz, 2H), 7.20 (d, $J$ = 8.0 Hz, 2H), 6.94 (br t, $J$ = 5.4 Hz, 1H), 3.79 (t, $J$ = 4.8 Hz, 2H), 3.59 (q, $J$ = 4.8 Hz, 2H), 3.25 (s, 3H), 3.17 (s, 3H), 3.03 (d, $J$ = 12.0 Hz, 1H), 2.14 (q, $J$ = 12.0 Hz, 1H), 2.06 (m, 1H), 1.22 (s, 3H), 0.77 (d, $J$ = 6.4 Hz, 3H).

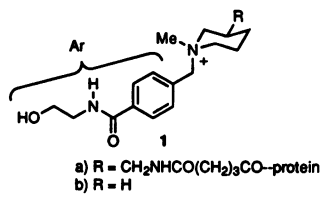
**Antibody-Catalyzed Hydrolysis.** All reactions were carried out using antibody 14D9 (ref. 9) (5 $\mu$M), substrate (50–500 $\mu$M), sodium phosphate-buffered saline (50 mM, pH 7.4, 100 mM NaCl). Progress of the reaction was monitored at 254 nm by reverse-phase HPLC (Hitachi L-6200A equipped with an AS-2000 autosampler and an Asahipak ODP-50 column (25 cm × 4.6 mm, 5-$\mu$m beads), using an acetonitrile/water gradient at 0.8 ml/min.

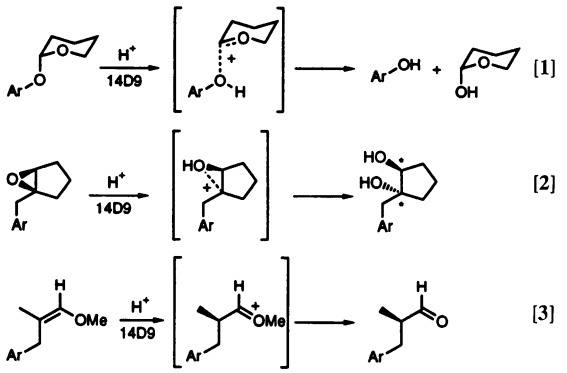
**Measurements of Kinetic Solvent Isotope Effect.** The hydrolysis of ketal **6a** was carried out as described above, at pH 7.55, 0°C, in either $H_2O$ or $^2H_2O$ [95% $^2$H (D)]. The pD values were obtained by adding 0.4 to the pH electrode readings.

**Measurements of Brønsted Coefficients.** Following the work of Kresge *et al.* (8), rates of hydrolysis of **2**, **4**, and **6a** (0.25 mM) were measured at 24°C in aqueous solutions containing [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane (Bistris) buffer (50 mM, pH 7.0) and one of the following: (*i*) NaCl (100 mM), (*ii*) NaCl (50 mM) and $RCO_2Na$ (50 mM), (*iii*) $RCO_2Na$ (100 mM). The following sodium carboxylates were used (R, p$K_a$): $CNCH_2$, 2.47; $ClCH_2$, 2.87; $CH_3OCH_2$, 3.57; H, 3.75; $HOCH_2$, 3.83; $CH_3$, 4.76; and $CH_3CH_2$, 4.88. All solutions were then adjusted to pH 7.0. Product formation in these solutions of identical pH was monitored by HPLC and the data were interpreted in terms of first-order kinetics. In the case of **2**, the observed rate enhancement in solutions *ii* and *iii*, as compared with *i*, varied from 1.25- (with 50 mM cyanoacetate) to 10-fold (with 100 mM propionate) and was found to be directly proportional to the concentration of the carboxylate. The Brønsted coefficient (0.67) was obtained by plotting the logarithm of the rate constant for each carboxylic acid vs. its pKa. In the case of ketals **4** and **6a** no measurable rate enhancement could be detected in reactions carried out under the same set of conditions.

## RESULTS AND DISCUSSION

Monoclonal antibodies which have been elicited against hapten **1a** were found to catalyze acid-promoted hydrolytic reactions, such as cleavage of a cyclic acetal (Eq. **1**) (9), enantioselective hydrolysis of an epoxide (Eq. **2**) (10), and enantioselective protonolysis of an enol ether (Eq. **3**) (11).




We have recently presented evidence that catalysis of the latter reaction by antibody 14D9 occurs via stabilization of the intermediate oxocarbonium ion by an ionizable protein side chain (7, 10). Hydrolysis of both enol ethers **2** and **3** (Fig. 1), which proceeds via the same intermediate **I**, was found to be catalyzed by this antibody (7). Under acid-catalyzed hydrolysis the related ketal **4** probably shares the same oxocarbonium intermediate **I**. As hydrolyses of both enol ethers and ketals are known to have late transition states (12–14) we expected that **4** should be a suitable substrate for antibody 14D9. Nevertheless, despite the fact that the rate of hydrolysis of **4** in water is greater than that of **2** and **3**, no measurable catalysis could be detected with this ketal (Fig. 1). The order of reactivity of **2**, **3**, and **4** 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 to the behavior of **4**, the two less rigid ketals **6a** and **6b** are catalytically hydrolyzed by 14D9 (Table 1). This enzyme-like catalysis is evident from the observed Michael-

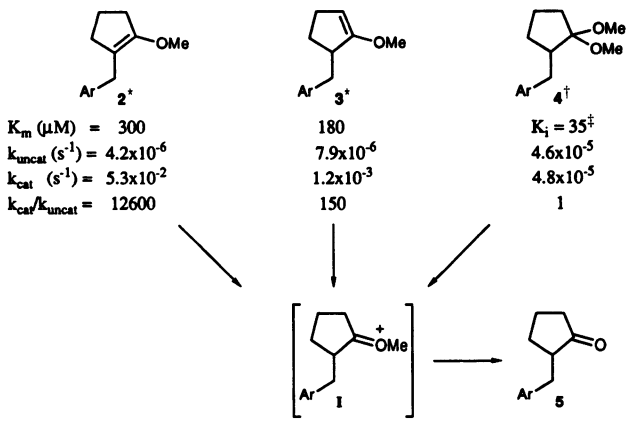
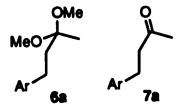
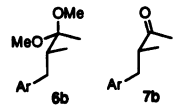
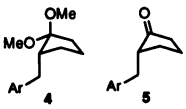



FIG. 1. 14D9-catalyzed hydrolysis of **2**, **3**, and **4**.
*From ref. 7, measured in 50 mM Bistris/100 mM NaCl, pH 7.0, at 20°C.
†This work, measured under the same conditions.
‡Inhibition constant for competitive inhibition of 14D9-catalyzed hydrolysis of **6a**, measured in 50 mM phosphate/100 mM NaCl, pH 7.55, at 0°C.

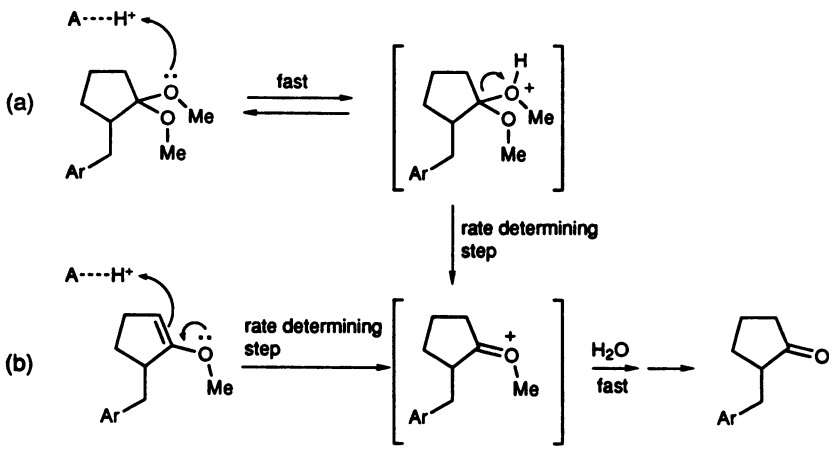
Table 1. Kinetic data for 14D9-catalyzed hydrolysis of ketals **6a**, **6b**, and **4**

| Substrate | Product | $K_m$, μM | $k_{uncat}$, $s^{-1}$ | $k_{cat}$, $s^{-1}$ | $k_{cat}/k_{uncat}$ |
|---|---|---|---|---|---|
|  | | 230 | $4.2 \times 10^{-7}$ | $1.8 \times 10^{-4}$ | 430 |
|  | | 160<br>$K_i$* = 60 μM | $6.9 \times 10^{-7}$ | $1.6 \times 10^{-5}$ | 23 |
|  | | $K_i$* = 35 μM | $1.8 \times 10^{-6}$ | $2 \times 10^{-6}$ | 1† |

All reactions were carried out at 0°C in 50 mM phosphate/100 mM NaCl at pH 7.55 and 0°C.
*Inhibition constants ($K_i$) for competitive inhibition of 14D9-catalyzed hydrolysis of **6a** were measured in 50 mM phosphate/100 mM NaCl at pH 7.55 and 0°C.
†No rate enhancement could be detected.

is–Menten kinetics and from the fact that catalysis is totally inhibited in the presence of stoichiometric quantities (with respect to 14D9) of the inhibitor **1b** (10). At 0°C and pH 7.55, the order of reactivity of the three chemically similar ketals **6a**, **6b**, and **4** toward hydrolysis is 0.23:0.38:1. However, this order is reversed to 100:9:1 within the antibody active site. Remarkably, all three ketals show very similar affinity to 14D9. The $K_m$ values of substrates **6a** and **6b** are comparable (Table 1). Moreover, ketal **4**, which shows no measurable catalysis, acts as a competitive inhibitor of 14D9-catalyzed hydrolysis of **6a** with $K_i$ = 35 μM. The corresponding $K_i$ value for **6b** is 60 μM (Fig. 2).

The hydrolysis of enol ethers, such as **2** and **3**, 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 Scheme II) (8). By contrast, hydrolysis of ketals, such as **4**, 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 Scheme II). Thus, the hydrolysis rates of **4**, **6a**, and **6b** are not affected by general acids and depend on the pH only. Accordingly, the selectivity of antibody 14D9 for **2** and **3** over **4** could be attributed to the presence of a protein side chain acting as a general acid in the active site. However, the observation of a catalytic reaction with ketals **6a** and **6b** clearly demonstrates that 14D9 can hydrolyze ketals closely related to **4** 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 **6a**: $(k_D/k_H)_{cat}$ = 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 Scheme II). 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 (15). 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, for which $k_D/k_H$ = 1.33 was measured for the hydronium ion catalysis (15)—or when the oxocarbonium intermediate is highly stable—e.g., in the case of tropone diethyl ketal (13). 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 **4** and **6a** between pH 6.0 and 10.0 {50 mM 1,3-bis[tris(hydroxymethyl)methylamino]propane (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




Scheme II

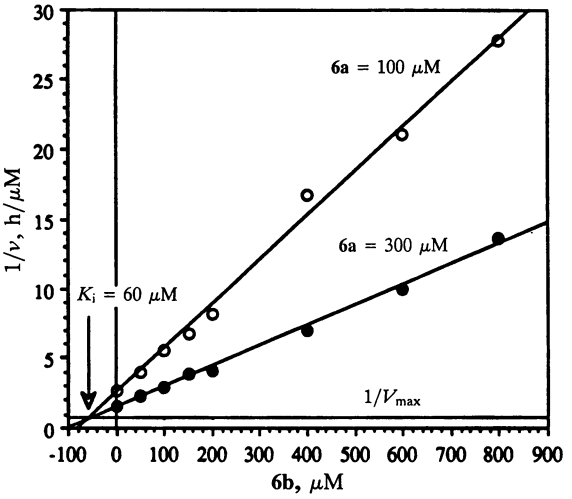



FIG. 2. Competitive inhibition of 14D9-catalyzed hydrolysis of **6a** with ketal **6b**. All reactions were carried out at 0°C in 50 mM phosphate/100 mM NaCl at pH 7.55.

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.

## CONCLUSION

The ability to control chemoselectivity by chemical catalysis represents one of the chemist's main goals. We presented here two examples of reversal of chemoselectivity that were achieved under antibody catalysis: (*i*) activation of enol ethers in the presence of a ketal and (*ii*) 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 simply be 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 future design of selective biocatalysts.

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1. Lerner, R. A., Benkovic, S. J. & Schultz, P. G. (1991) *Science* **252,** 659–667.
2. Hsieh, L. C., Yonkovich, S., Kochersperger, L. & Schultz, P. G. (1993) *Science* **260,** 337–339.
3. Keinan, E., Sinha, S. C. & Sinha-Bagchi, A. (1991) *J. Chem. Soc. Perkin Trans.* **1,** 3333–3339.
4. Katritzky, A. R., Rachwal, S., Rachwal, B. & Steel, P. J. (1992) *J. Org. Chem.* **57,** 4925–4931.
5. Faunce, J. A., Grisso, B. A. & Mackenzie, P. B. (1991) *J. Am. Chem. Soc.* **113,** 3418–3426.
6. Trost, B. M. (1980) *Acc. Chem. Res.* **13,** 385–393.
7. Reymond, J.-L., Jahangiri, G. K. & Lerner, R. A. (1993) *J. Am. Chem. Soc.* **115,** 3909–3917.
8. Kresge, A. J., Chen, H. L., Chiang, Y., Murrill, E., Payne, M. A. & Sagatys, D. S. (1971) *J. Am. Chem. Soc.* **93,** 413–423.
9. Reymond, J.-L., Janda, K. D. & Lerner, R. A. (1991) *Angew. Chem. Int. Ed. Engl.* **30,** 1711–1713.
10. Sinha, S. C., Keinan, E. & Reymond, J.-L. (1993) *J. Am. Chem. Soc.* **115,** 4893–4894.
11. Reymond, J.-L., Janda, K. D. & Lerner, R. A. (1992) *J. Am. Chem. Soc.* **114,** 2257–2258.
12. Kresge, A. J., Sagatys, D. S. & Chen, H. L. (1977) *J. Am. Chem. Soc.* **99,** 7228–7233.
13. Anderson, E. & Fife, T. H. (1969) *J. Am. Chem. Soc.* **91,** 7163–7166.
14. Kirby, A. J. (1987) *CRC Crit. Rev. Biochem.* **22,** 283–315.
15. Fife, T. H. & Jao, L. K. (1968) *J. Am. Chem. Soc.* **90,** 4081–4085.