Despite the above observations, which are consistent with a mechanism-based inactivation,[20] *a spontaneous, temperature-dependent reactivation of BPAO occurred at later times* (see, e.g., Figure 1 plot without added salt).[21] This recovery is much faster than that observed in the AMTMS inactivation of MAO, for which an active site trimethylsilylation was initially considered, but which was ultimately shown through elegant kinetics and labeling experiments to involve enzyme attack on a $Me_3SiCH{=}NH_2^+$ species.[2] The rapid recovery seen with BPAO is not inconsistent with the expected hydrolytic lability of a trimethylsilylated heteroatom, which might be potentiated by F^-. In Figure 1, we show that 0.4 M KF alters the time scale of the interaction of AMTMS with BPAO at pH 9.0 no more than that seen with 0.4 M KCl.[22] Although this result fails to support an active-site trimethylsilylation, F^- could be ineffective on account of its tight hydration in aqueous medium and/or its inability to penetrate to the site of covalent modification.

Additional work will be required to clarify the nature of interaction of AMTMS with BPAO, including the use of α-deuterated AMTMS[2] to distinguish whether HCHO results from path A (eq 1) or path B with subsequent Brook rearrangement,[23] as well as studies using radiolabeled AMTMS and purified enzyme preparations. Nonetheless, the notion of an active-site-directed silylation, considered previously for MAO[2] and for inactivation of cytochrome $P\text{-}450_{scc}$ by a Me_3Si-containing steroid,[24] constitutes an intriguing protein modification tactic which may find important enzymologic applications using AMTMS-like agents.

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Supplementary Material Available: Experimental procedures and additional kinetic plots (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(20) Alternatively, there is a chance that AMTMS is acting as a "slow substrate" (k_i is then just k_{on}), the recovery of enzyme activity resulting from eventual turnover to noninhibiting products.

(21) The recovery of activity for enzyme inactivated with 100 μM AMTMS occurred with rates of 0.0099, 0.020, and 0.036 min^{-1} at 5, 10, and 30 °C.

(22) No alteration of the activity–time profile was observed using lower concentrations of KF or KCl and/or when the reactions were conducted at lower pH (7.2). Our observations using 0.4 M salts may represent an ionic strength effect.

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An Improved Procedure for the Synthesis and Use of $[RuCl_2(BINAP)]_2 \cdot NEt_3$. Dependence of the Ru(II)-BINAP Catalyzed Asymmetric Hydrogenation of β-Keto Esters on Trace Amounts of Acid

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Summary: The asymmetric hydrogenation of β-keto esters using 0.02–0.05 mol % Ru(II)–BINAP-derived catalyst can be conducted in a stardard Parr shaker apparatus at 40 °C and 30 psi of hydrogen in the presence of 0.1 mol % of a strong acid.

Asymmetric hydrogenation using the Ru(II)–BINAP system introduced by Noyori and co-workers provides very high enantioselectivity over a wide range of substrates with remarkable turnover.[1] However, all reports concerning the reduction of β-keto esters[2] suffer from the need for temperatures greater than 80 °C or hydrogen pressures greater than 1000 psi (80 atm) where special apparatus is required.[3] We communicate here that, in the presence of trace amounts of strong acid, asymmetric hydrogenation proceeds in a standard Parr shaker apparatus at low temperatures and readily attainable pressures (40 °C/30 psi H_2) with substrate/catalyst ratios up to 10 000. Additionally, we describe the kinetics of the reaction and a simple, reproducible procedure for preparation of purified catalyst.[3b,c]

The catalyst $[RuCl_2(BINAP)]_2 \cdot NEt_3$[4] is easily prepared using standard anaerobic benchtop techniques[3b,c] from commercially available (cyclooctadiene)ruthenium dichloride and (*R*)- or (*S*)-BINAP.[5] Filtration of the product using a double ended filter available from Kontes glass company provides a large measure of purification without the use of an inert atmosphere glovebox and avoids the need to remove large volumes of a high-boiling solvent in vacuo. We have used this technique to prepare from 200 mg–20 g of catalyst at a time. Reduction rates using this material are reproducible to ±10% across several batches. Although the catalyst must be stored under an inert atmosphere to prolong shelf life, it can be conveniently handled in air.

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(3) For the most recent examples, see: (a) Kitamura, M.; Tokunaga, M.; Ohkuma, T.; Noyori, R. *Tetrahedron Lett.* **1991**, *32*, 4163. (b) Taber, D. F.; Silverberg, L. J. *Tetrahedron Lett.* **1991**, *32*, 4227. (c) Keck, G. E.; Murry, J. A. *J. Org. Chem.* **1991**, *56*, 6606.

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(5) [RuCl(PhH)(BINAP)]Cl is also an effective catalyst available in one step from commercially available materials: Mashima, K.; Kusano, K.; Ohta, T.; Noyori, R.; Takaya, H. *J. Chem. Soc., Chem. Commun.* **1989**, 1208. See also ref 3a. We have avoided this catalyst due to its benzene content.

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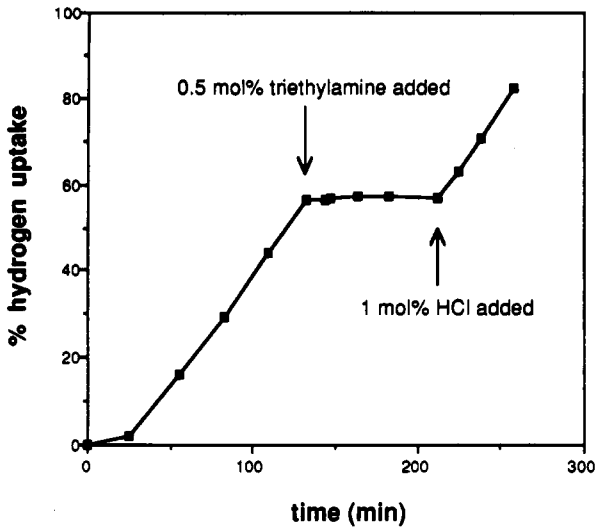
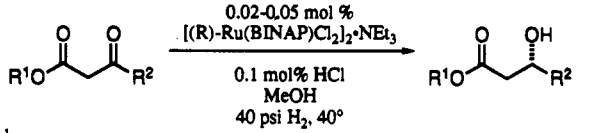


Figure 1. Percent of theoretical hydrogen uptake versus time at 42 °C/150 psi, 0.04 mol % catalyst.

We routinely conduct asymmetric reductions at catalyst levels of 0.02–0.05 mol %. The orange-red catalyst is added to a solution of the β-keto ester in methanol (0.5–2.25 M) which has been deoxygenated for several minutes with flowing nitrogen. At this point 0.1 mol % of a strong acid is added (vide infra). We have conducted reductions at pressures from 30 to 150 psi and at temperatures of 40–60 °C. Reductions are complete in 3–8 h. Preparation of the Mosher ester of the product indicates that the enantiomeric excess is 97+% in all cases.



R^1= CH_3, t-Bu
R^2= CH_3, $CH_2CH_2CH_2OCH_3$

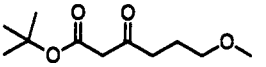
In order to test the mildness of our conditions, we chose *tert*-butyl acetoacetate as our standard substrate.[6] This class of compounds, whose ester function is easily deprotected in the presence of acid, is transesterified under purely thermal conditions (at 70 °C, 12% transesterification is observed in 1 h).[7] Under our reduction conditions only 3% of the methyl ester is produced.

The reaction shows interesting kinetics.[8] While there is an expected first-order dependence on catalyst and hydrogen, the reaction is zero order in keto ester. After an initial 20-min period during which the catalyst dissolves, hydrogen uptake is linear with time. This kinetic profile is probably indicative of a rate-limiting hydrogen activation step[9] which precedes substrate binding and hydrogen transfer, which are fast.

Our most interesting observation is a dramatic dependence of the reaction upon low levels of strong acid.[3b] In one case, a reaction mixture containing no acid was exposed to 50 psi of hydrogen at 50 °C for 24 h with no hydrogen uptake. When 1 mol % HCl was added, the reaction went to completion in 3 h.[10] Sulfuric acid was equally effective. Significantly, the catalyst [RuCl(PhH)(BINAP)]Cl,[5] which does not contain endogenous amine, also shows this acid dependence.[11] Because kinetics experiments show that the reaction is zero order in $[H^+]$ even at 5×10^{-4} M, we now routinely use 0.1 mol % HCl in our reaction mixtures. Thus, a very low concentration of acid is required for maximal reaction rate; any further increase in acid concentration provides no rate enhancement.

The effect of added acid is reversible. As illustrated in Figure 1, hydrogen uptake is immediately halted upon addition of 0.5 mol % triethylamine (pK_a = 11) to a progressing reduction (42 °C/150 psi) containing 0.1 mol % HCl. The reaction proceeds at its original rate again upon reacidification. At 42 °C/50 psi, 0.5 mol % of the weaker base 2,6-di-*tert*-butylpyrididine (pK_a = 5) also stops the reaction; however, at 150 psi only a 70% rate reduction is observed. The reports[1,3a,c] that pressures in excess of 1000 psi are required for β-keto ester reduction may be a result of this attenuation of the pH effect at higher pressures. The effect of such a very hindered base on the rate of hydrogenation rules out the possibility that coordination of the amine to the catalyst is important.

Our experience is that substrates which are unwilling to hydrogenate probably contain low-level basic impurities. The keto ester 1, prepared by acetoacetic ester dianion



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alkylation, hydrogenated well under the usual conditions when carefully distilled. However, when a short-path apparatus was used, the level of added acid had to be increased to 0.8–1.8 mol %. The reaction then proceeded at its usual rate with the normal amount of transesterification with the solvent. We have also observed that some catalyst batches which have been stored for long periods require no exogenous acid for reaction. ^1H NMR shows that triethylamine signals move downfield over time, indicating that the catalyst may become acidic upon decomposition.

Catalyst Preparation. (Cyclooctadienyl)ruthenium dichloride (214 mg, 0.76 mmol) and (*R*)-BINAP (500 mg, 0.80 mmol) were placed in a 50-mL round-bottom flask and connected to a double-ended filter (Kontes No. 215500-6044) with a 100-mL round-bottom flask at the opposite end. Vacuum grease was used to ensure an air-tight seal. Rubber bands were a simple and effective way of holding the apparatus together. The entire apparatus was evacuated and filled with nitrogen. Toluene (17 mL) and triethylamine (1.7 mL), which had been deoxygenated with flowing nitrogen for several minutes, were added via the lower side arm. The vessel was sealed[12] and the mixture heated to 140 °C producing a deep brick red colored solution.[13] After 4 h the apparatus was allowed to cool to room temperature with vigorous stirring while the catalyst precipitated. The apparatus was vented to nitrogen and inverted to filter the product using vacuum on the lower

(6) Witzeman, J. S.; Nottingham, W. D. *J. Org. Chem.* **1991**, *56*, 1713. The asymmetric reduction does not proceed in 2-methyl-2-propanol.

(7) While most of our work used this substrate the principle conclusions were confirmed using methyl acetoacetate and keto ester 1.

(8) A detailed study of the asymmetric reduction of acrylates by Ru(II)–BINAP catalysts have been performed: Ashby, M. T.; Halpern, J. *J. Am. Chem. Soc.* **1991**, *113*, 589.

(9) Halpern, J.; Harrod, J. F.; James, B. R. *J. Am. Chem. Soc.* **1966**, *88*, 5150.

(10) It is interesting that Halpern (ref 8) has noted the reduction of acrylic acids is halted by the presence of strong acids.

(11) For some interesting observations concerning reactions of this catalyst in methanol, see: Mashima, K.; Hino, T.; Takaya, H. *Tetrahedron Lett.* **1991**, *32*, 3101. Our NMR work has not shown the presence of the ruthenium trimer reported by these workers.

(12) Alternatively, the reaction can be conducted using xylene as solvent. In this case the catalyst is prepared in a three-neck flask with a reflux condenser and the two-way filter. This method is most convenient on a 5–20-g scale.

(13) If the red solution is not produced then oxygen is most likely present.

side arm and nitrogen on the upper. If the frit caked with product and filtration slowed, momentary reversing of the flow of nitrogen lifted the cake away from the frit. The precipitate was washed with deoxygenated toluene (17 mL), and the flask containing the filtrate was exchanged for an empty one. (^{31}P NMR showed that the filtrate contained none of the desired product.) The entire apparatus was put under vacuum, and the product was dried overnight to give 470 mg (75%) of a dark red solid which appeared orange when pulverized: ^{1}H NMR (CD_2Cl_2, 101 MHz) δ 8.07 (t, J = 8.8 Hz, 4 H), 7.82 (t, J = 8.3 Hz, 2 H), 7.65 (t, J = 8.3 Hz, 6 H), 7.55 (t, J = 8.8 Hz, 4 H), 7.47 (dd, J = 11.2, 8.8 Hz, 4 H), 7.4–7.1 (m, 20 H), 6.95 (t, J = 7.5 Hz, 2 H), 6.84 (t, J = 7.4 Hz, 2 H), 6.8–6.7 (m, 4 H), 6.7–6.6 (m, 4 H), 6.6–6.5 (m, 12 H), 3.24 (m, 6 H), 1.45 (t, J = 7.3 Hz, 9 H); ^{31}P NMR (CD_2Cl_2, 101 MHz) δ 56.5 (d, J = 38 Hz), 52.3 (d, J = 38 Hz).

Asymmetric Reductions. *tert*-Butyl acetoacetate (14.5 g, 90 mmol) and methanol (30 mL) were mixed and deoxygenated with flowing nitrogen for 5 min in a septum-covered Parr shaker bottle. The catalyst prepared above (36 mg, 0.041 mmol) was added along with 2 N HCl (0.041 mL, 0.082 mmol). The mixture was transferred to a standard Parr shaker apparatus and flushed by evacuating and refilling with nitrogen and then hydrogen several times. The apparatus was heated at 40 °C with shaking under 50 psi of hydrogen. After 20 min the reaction became a homogeneous clear yellow solution which took up hydrogen for approximately 8 h. At this time the reaction was complete and the mixture was cooled and diluted with hexane (30 mL) to precipitate the catalyst, which was filtered away. The filtrate was concentrated to give *tert*-butyl 3(*R*)-hydroxybutyrate (14.5 g, 97%).

Acknowledgment. We wish to acknowledge the very helpful assistance of Mr. William Benning of the Merck high-pressure laboratory. We thank Professor Taber for providing a preprint of his paper.

Supplementary Material Available: Additional procedures, reduction rates, and NMR spectra (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Antibody Catalysis in Low Water Content Media

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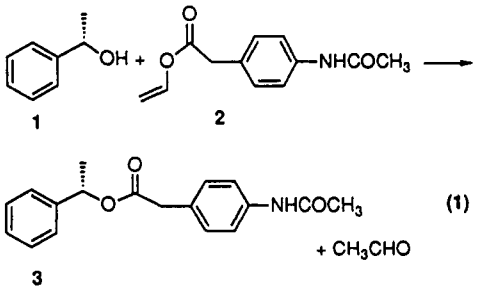
Summary: An investigation has been performed wherein an immunoglobulin's catalytic function in aqueous–organic biphasic solvent systems was elucidated, and the water requirements necessary to preserve its catalytic competency in an apolar solvent system were determined.

Enzymes can act as catalysts in organic solvents.[1] On the other hand, antibodies have only recently been investigated for their ability to function in organic media.[2] Lately we have focused our efforts on trying to observe antibody catalysis in organic solvents[3] in an effort to make catalytic antibodies more attractive catalysts from a synthetic standpoint. To take full advantage of the possible opportunities afforded by catalytic antibodies[4] in organic solvents it is critical to understand their "abzymology" when they are subjected to these nonnatural environments. Herein, we report on an immunoglobulin's catalytic activity in a variety of apolar solvent systems and elucidate its water requirements in such media.

It has been shown that an antibody can catalyze a hydrolysis reaction in a reverse micelle.[5] However, there have been no reports of abzymes functioning at an aqueous-organic interface or in low water containing media. Previously, we studied a set of antibodies which stereoselectively hydrolyzed either the (*R*) or (*S*) enantiomer of an alkyl ester to its acid and α-methyl benzyl alcohol components.[6] One of these abzymes (PCP 21H3) could catalyze an enantioselective acyl transfer reaction (eq 1)



(1)

via a ping-pong mechanism.[7] However, our attempts to catalyze this transesterification reaction in polar organic solvents proved unsuccessful.[3]

It was quickly determined that octane (log P = 4.5)[8] was an excellent solvent system for the abzyme's catalysis. A

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